PCT

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07C 69/34, 229/24, 233/04 · C07C 235/20, A61K 31/22	**A1**	(11) International Publication Number: **WO 91/08191** (43) International Publication Date: 13 June 1991 (13.06.91)

(21) International Application Number: PCT/US90/06649

(22) International Filing Date: 14 November 1990 (14.11.90)

(30) Priority data:
PCT/US89/05203   14 November 1989 (14.11.89) WO
*(34) Countries for which the regional
or international application
was filed:*   US et al.
522,558   14 May 1990 (14.05.90)   US
PCT/US90/02690 14 May 1990 (14.05.90)   WO
*(34) Countries for which the regional
or international application
was filed:*   US et al.

(60) Parent Application or Grant
(63) Related by Continuation
US   522,528 (CIP)
Filed on   14 May 1990 (14.05.90)

(71) Applicants *(for all designated States except US)*: SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH [US/US]; 1275 York Avenue, New York, NY 10021 (US). THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK [US/US]; West 116th Street & Broadway, New York, NY 10027 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : MARKS, Paul, A. [US/US]; Beach Hill Road, Bridgewater, CT 06752 (US). RIFKIND, Richard, A. [US/US]; 30 Sutton Place, New York, NY 10022 (US). BRESLOW, Ronald [US/US]; 275 Broad Avenue, Englewood, NJ 07631 (US). JURSIC, Branko [US/US]; 100 Morningside Drive, Apt. 3F, New York, NY 10027 (US).

(74) Agent: WHITE, John, P.; Cooper & Dunham, 30 Rockefeller Plaza, New York, NY 10112 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK, DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: NOVEL POTENT INDUCERS OF TERMINAL DIFFERENTIATION AND METHOD OF USE THEREOF

(57) Abstract

   The invention provides compounds, several of which belong to a class having two or more nonpolar components connected by a polar group and having polar groups on the termini of the compound. The invention also concents a method of selectively inducing termini differentiation of neoplastic cells and thereby inhibiting proliferation of such cells which comprises contacting the cells under suitable condition with an amount of the compound effective to selectively induce terminal differentiation. Moreover, the invention provides a method of treating a patient having a tumor characterized by proliferation of neoplastic cells which comprises administering to the patient an amount of the compound effective to selectively induce terminal differentiation of such neoplastic cells, thereby inhibiting their proliferation and suppressing oncogenicity. Lastly, the present invention provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and the compound in an amount effective less than an amount which would cause toxicity in the patient.

-1-

NOVEL POTENT INDUCERS OF
TERMINAL DIFFERENTIATION AND METHOD OF USE THEREOF

This is a continuation-in-part application of U.S. Serial No. 522,558, filed May 14, 1990, which in turn is a continuation-in-part application of U.S. Serial No. 374,343, filed June 30, 1989, which in turn is a continuation-in-part of application U.S. Serial No. 270,963, filed November 14, 1988, the contents of which are hereby incorporated by reference into the present application.

This application also claims the benefit of PCT International Application No. PCT/US90/02690, filed May 14, 1990 and International Application No. PCT/US89/05203, filed November 14, 1989, under 35 U.S.C. §119.

Background of the Invention:

Throughout this application various publications are referenced by arabic numerals within parentheses. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Cancer is a disorder in which a population of cells has become, in varying degrees, unresponsive to the control mechanisms which normally govern proliferation and differentiation. For many years there have been two principal strategies for chemotherapeutic treatment of cancer: a) blocking hormone-dep nd nt tumor cell proliferation by interference with th production or peripheral action f sex hormones; and b) killing cancer

-2-

cells directly by exposing them to cytotoxic substances,
which injure both neoplastic and normal cell populations.

Relatively recently, cancer therapy is also being attempted
by the induction of terminal differentiation of the
neoplastic cells (1). In cell culture models
differentiation has been reported by exposure of cells to a
variety of stimuli, including: cyclic AMP and retinoic acid
(2,3), aclarubicin and other anthracyclines (4).

There is abundant evidence that neoplastic transformation
does not necessarily destroy the potential of cancer cells
to differentiate (1,5,6). There are many examples of tumor
cells which do not respond to the normal regulators of
proliferation and appear to be blocked in the expression of
their differentiation program, and yet can be induced to
differentiate and cease replicating. A variety of agents,
including some relatively simple polar compounds (5,7-9),
derivatives of vitamin D and retinoic acid (10-12), steroid
hormones (13), growth factors (6,14), proteases (15,16),
tumor promoters (17,18), and inhibitors of DNA or RNA
synthesis (4,19-24), can induce various transformed cell
lines and primary human tumor explants to express more
differentiated characteristics.

Early studies by the present inventors identified a series
of polar compounds that were effective inducers of
differentiation in a number of transformed cell lines (8,9).
Of these, the most effective inducer, until recently, was
the hybrid polar/apolar compound N,N'-hexamethylene
bisacetamide (HMBA) (9). The use of polar/apolar compounds
to induce murine erythroleukemia cells (MELC) to undergo
erythroid differentiation with suppression of oncogenicity
has proved a useful mod l to study inducer-mediated
diff r ntiation of transformed cells (5,7-9). HMBA-induced

MELC terminal erythroid differentiation is a multistep process. Upon addition of HMBA to MELC (745A-DS19) in culture, there is a latent period of 10 to 12 hours before commitment to terminal differentiation is detected. Commitment is defined as the capacity of cells to express terminal differentiation despite removal of inducer (25). Upon continued exposure to HMBA there is progressive recruitment of cells to differentiate. Recently, the present inventors reported that MELC cell lines made resistant to relatively low levels of vincristine become markedly more sensitive to the inducing action of HMBA and can be induced to differentiate with little or no latent period (26).

HMBA is capable of inducing phenotypic changes consistent with differentiation in a broad variety of cells lines (5). The characteristics of the drug induced effect have been most extensively studied in the murine erythroleukemia cell system (MELC) (5,25,27,28). MELC induction of differentiation is both time and concentration dependent. The minimum concentration required to demonstrate an effect in vitro in most strains is 2 to 3 mM; the minimum duration of continuous exposure generally required to induce differentiation in a substantial portion (>20%) of the population without continuing drug exposure is about 36 hours.

The primary target of action of HMBA is not known. There is evidence that protein kinase C is involved in the pathway of inducer-mediated differentiation (29). The in vitro studies provided a basis for evaluating the potential of HMBA as a cytodifferentiation agent in the treatment of human cancers (30). Several phase I clinical trials with HMBA have been completed (31-36). R cently, the first evidence was r p rted that this comp und can induce a therap utic

-4-

response in patients with cancer (35,36). These phase I clinical trials demonstrate that the potential efficacy of HMBA is limited, in part, by dose-related toxicity which prevents achieving optimal blood levels and by the need for intravenous administration of large quantities of the agent, over prolonged periods.

The present invention provides new chemical inducers which are many times more active than HMBA. It has unexpectedly been found that compounds having two or more nonpolar components connected by a polar group and having groups on the termini of the compound are effective inducers of terminal differentiation. For instance, bis-hexamethylene triacetamide, which comprises three acetamide groups connected by two six-carbon chains, has been found to be a potent inducer of terminal differentiation in MELC.

This new class of compounds of the present invention may be useful for selectively inducing terminal differentiation of neoplastic cells and therefore aid in treatment of tumors in patients.

-5-

Summary of the Invention:

The invention provides a class of compounds having the structure:
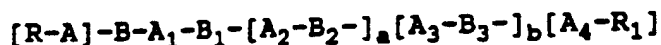
$$[R-A]-B-A_1-B_1-[A_2-B_2-]_a[A_3-B_3-]_b[A_4-R_1]$$

wherein each of A, A_1, A_2, A_3, and A_4 represent a polar group which comprises a nitrogen, sulfur or oxygen atom wherein each of A, A_1, A_2, A_3, and A_4 may independently be the same as, or different from, the others of A, A_1, A_2, A_3, and A_4;

wherein each of R and R_1 is a hydrogen atom; a lower alkyl, alkenyl, or alkynyl group; or a group having the structure:

$$\begin{array}{c} R_2 \\ | \\ -N \\ | \\ R_3 \end{array}$$
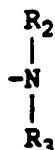
wherein each of R_2 and R_3 being a hydrogen atom or a lower alkyl, alkenyl, or alkynyl group; and wherein each of R, R_1, R_2 and R_3 may independently be the same as, or different from the other of R, R_1, R_2 and R_3;

wherein each of [R-A] and [A_4-R_1] have a dipole moment greater than about 2.7 Debye units;

wherein each of B, B_1, B_2, and B_3 represents a nonpolar group which comprises at least 4 atoms in a chain, the termini of which chains are attached to A and A_1, A_1 and A_2, A_2 and A_3, and A_3 and A_4, respectively; wherein each such atom is oxygen, nitrogen, carbon, or sulfur and wherein each of B, B_1, B_2, and B_3 may ind pendently be the same as, or

-6-

different from the others of B, B_1, B_2 and B_3;

and wherein each of a and b is independently 0 or 1.

Other compounds of this invention include a compound having the structure:

$$CH_3-N \quad N-CH_3$$

$$(CH_3)_2N-\overset{O}{\overset{\|}{C}}-(CH_2)_5 \quad (CH_2)_5-\overset{O}{\overset{\|}{C}}-N(CH_3)_2 \; ;$$

or a compound having the structure:

$$R_1-O-\overset{O}{\overset{\|}{C}} \quad \overset{O}{\overset{\|}{C}}-O-R_2$$

$$H3C-N-\overset{}{\underset{H3C}{\overset{}{\underset{O}{C}}}}-(CH_2)_3 \quad (CH_2)_3-\overset{}{\underset{O}{\overset{}{\underset{CH_3}{C}}}}-N-CH_3$$

wherein R1 and R2 may be the same or different and each is a lower alkyl group;

Also provided by this invention is a compound having the structure:

$$CH_3-N \quad N-CH_3$$

$$R \quad (CH_2)-\overset{}{\underset{O}{\overset{}{C}}}-N-(CH_3)_2$$

wherein R is H or a lower alkyl group.

-7-

This invention further provides a compound having the structure:

5

10

wherein n is an integer which is greater than 1 and R_1 and R_2 may be the same or different and each is H or a lower alkyl group.

15

A compound having the structure:

20

25

is also provided by this invention wherein X1 and X2 may independently be the same or different and each is N(CH3)2 NH-phenyl, or HNCH3.

30

35

-8-

This invention also provides a compound having the structure:

$$\text{OH-N-C-(CH}_2)_6\text{-C-N-OH}$$

with H3C O above OH-N-C and O CH3 above C-N-OH

5

or a compound having the structure:

10

$$2((CH3)2\text{-N-C)-C-(CH2)n-C-(C-N-(CH3)2))2}$$
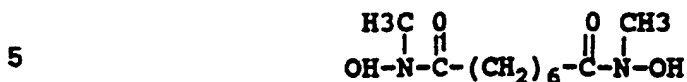
with O R1 ... R2 O above
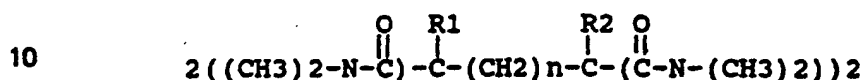
wherein R1 and R2 may be the same or different and each is H or a lower alkyl group; and n is an integer from 1 to 10.

15   This invention further provides a compound having the structure:
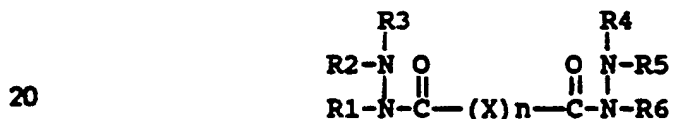
20

$$\text{R1-N-C——(X)n——C-N-R6}$$

with R3, R2-N O above left and R4, O N-R5 above right

wherein R1, R2, R3, F4, R5, R6 may independently be the same or different from each other and is H or a lower alkyl group; wherein X is methyl or phenyl; and n is an integer
25   from 1 to about 15.

A compound having the structure:

30

R2-N —————— N-R1
O=C   C=O
        C
H3C O   O CH3
CH3-N-C-(CH2)5   (CH2)5-C-N-CH3

35
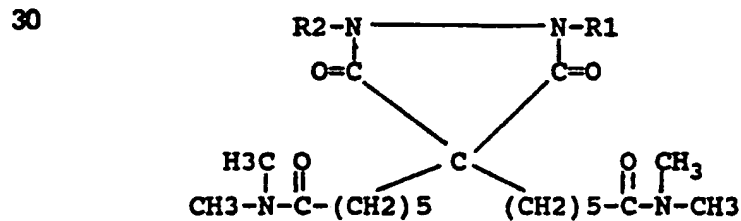
-9-

is also provided by this invention wherein R1 and R2 may be the same or different and is H or CH3.

The invention also provides a compound having the structure:

$$_2HC \underline{\hspace{2cm}} CH_2$$
$$R2-N \qquad\qquad N-R1$$
$$C \qquad C$$
$$O \qquad O$$
$$C$$
$$CH_3 - N - C-(CH_2)_5 \qquad\qquad (CH2)5-C-N-CH3$$
$$\qquad\quad CH3\ O \qquad\qquad\qquad O\ CH3$$
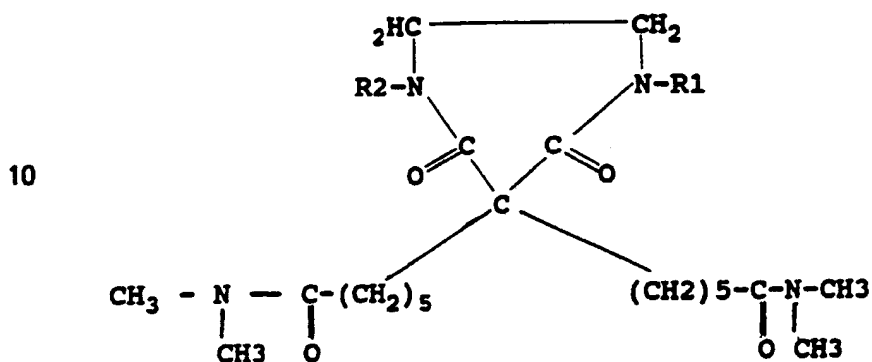
wherein R_1 and R_2 may be the same or different and is H or CH_3.

Further provided is a compound having the structure:

$$\begin{matrix} R_1 & O & & O & R_2 \\ | & \| & & \| & | \\ HO-N & -C- & X- & C & -N- OH \end{matrix}$$

wherein X has the structure:

$-CH=CH-\bigcirc-CH=CH-$,

$-C\equiv C-\bigcirc-C\equiv C-$,

$-\bigcirc-C\equiv C-$,

$-\bigcirc-CH=CH-$

$-\bigcirc-$

$-CH2-\bigcirc-CH2-$, or

$-\bigcirc-\bigcirc-$

$-CH2\underset{H}{\overset{H}{C}}=\overset{H}{\underset{CH_2}{C}}-$

$-CH=CH-CH=CH;$

wherein R_1 and R_2 are the same or different and is H or lower alkyl group.

Further provided by this invention is a compound having the structure:

$$\text{(CH}_2)\text{n-C-N-R3}$$

wherein R1 and R1 may independently be the same or different and each may be H or a lower alkyl group; wherein R3 and R4 may independently be the same or different and each may be CH3 or OH; and wherein n is 5 or 6.

A compound having the the structure:

$$(CH3)2-N-C-(CH2)6-C-(CH2)6-C-N-(CH3)2$$

is also provided by this invention, wherein R_1 and R_2 is the same or different and is hydrogen, lower alkyl, alkenyl, alkynyl, an amide or hydroxyamide.

This invention further provides compounds having the following structures:

-N(CH3)-CO-(CH2)5-CO-N(CH3)-

and

-11-

$$
\begin{array}{cc}
H5C2-O & O-C2H5 \\
| & | \\
O=C & C=O \\
\backslash & / \\
& C \\
/ & \backslash \\
HO-NH-CO-(CH2)5 & (CH2)5-CO-NH-OH
\end{array}
$$

also,

[benzene ring]—N(CH3)-CO—(CH2)5—CO-N(CH3)—[benzene ring]

and a compound having the structure:

$$
\begin{array}{cc}
C2H5 & H \\
| & | \\
O & N—[benzene ring] \\
| & | \\
O=C & C=O \\
\backslash & / \\
2(CH3)N-CO-(CH2)5-C-(CH2)5-CO-N(CH3)2
\end{array}
$$

The invention also concerns a method of selectively inducing terminal differentiation of neoplastic cells and thereby inhibiting proliferation of such cells which comprises contacting the cells under suitable condition with an effective amount of any of the compounds listed above effective to selectively induce terminal differentiation.

Moreover, the invention provides a method of treating a patient having a tumor characterized by proliferation of neoplastic cells which comprises administering to the patient an effective amount of any of the compounds of the subject invention in an amount effective to selectively induce terminal differentiation of such neoplastic cells, thereby inhibiting their proliferation, and suppressing oncogenicity.

Lastly, the present invention provides a pharmaceutical c mposition comprising a pharmac utically acceptabl carrier and any of the compounds of the subject inv ntion in an

-12-

amount less than an amount which cause toxicity in the patient.

5

10

15

20

25

30

35

Brief Description of the Figures

Figure 1:   Comparison of the hybrid polar/apolar
             compounds, (A) hexamethylene bisacetamide
             (HMBA); (B) suberic acid bis-N-methyl
             diacetamide (SBDA); and (C) bis-
             hexamethylene triacetamide (BHTA) as
             inducers of differentiation of vincristine-
             sensitive (745A-DS19) (●) and vincristine-
             resistant (VCR.C(2) 15) (▲) MELC for HMBA
             and SBDA and (V3.17) for BHTA. Each
             compound was added to the cells in culture
             at the final concentration indicated.
             Benzidine reactive cells (left panel of each
             section of this figure) were determined
             after 4 d. of culture and commitment (right
             panel of each section of this figure) after
             2 d. of culture.

Figure 2:   Concentration dependent curves of inducer
             activity of compound 12 with vincristine-
             sensitive (745A-DS19) (●) and vincristine-
             resistant (VCR.C(2)15) (▲) MELC. The
             compound was added to the cultures at the
             final concentrations indicated. Benzidine
             reactive cells were determined after 5 d. in
             culture and commitment after 2 d. of
             culture.

Figure 3:   Optimal concentration of IC-135 for
             inducement of terminal differentiation.

Figure 4:   Comparison of HMBA and IC-135 on DS19 cells.

-14-

Figure 5A
and B: Comparison of % cell committed for HMBA and
                        IC-135 on V3.17 and DS19 cell lines.

5   Figure
       6A and B: Comparison of B+% for HMBA and IC-135 on
                        V3.17 and DS19 cell lines.

10

15

20

25

30

35

-15-

Detailed Description of the Invention:

The invention provides a class of compounds having the structure:

$$[R-A]-B-A_1-B_1-[A_2-B_2-]_a[A_3-B_3-]_b[A_4-R_1]$$

wherein each A, A_1, A_2, A_3, and A_4 represent a polar group which comprises a nitrogen, sulfur or oxygen atom and wherein each of A, A_1, A_2, A_3, and A_4 may independently be the same as, or different from, the others of A, A_1, A_2, A_3, and A_4;

wherein each of R and R_1 is a hydrogen atom; a lower alkyl, alkenyl, or alkynyl group; or a group having the structure:

$$\begin{array}{c} R_2 \\ | \\ -N \\ | \\ R_3 \end{array}$$

wherein each of R_2 and R_3 being a hydrogen atom or a lower alkyl, alkenyl, or alkynyl group; and wherein each of R, R_1, R_2 and R_3 may independently be the same as, or different from, the others of R, R_1, R_2 and R_3;

wherein each of [R-A] and [A_4-R_1] have a dipolar moment greater than about 2.7 Debye units;

wherein each of B, B_1, B_2, and B_3 represents a nonpolar group which comprises at least 4 atoms in a chain, the termini of which chains are attached to A and A_1, A_1 and A_2, A_2 and A_3, and A_3 and A_4, respectively; wherein each such atom is xygen, nitrog n, carbon, or sulfur and wh rein each of B, B_1, B_2, and B_3 may independently be th same as, or differ nt from the others of B, B_1, B_2, and B_3;

-16-

and wherein each of a and b is independently 0 or 1.

The compounds of the present invention are made up of two components. One component comprises a polar group, i.e. functional groups with significant dipole moments, such as amides, sulfoxides, amine oxides and related functional groups.

The terminal portions of the compound, represented by R-A and A_4-R_1, each have dipole moments greater than about 2.7 debye units. The polar groups within the compound, represented by -A_1, -A_2- and -A_3-, have significant dipolar moments but not necessarily in excess of 2.7 debye units. In the preferred embodiments, the polar groups are carbonyl radicals or bivalent radicals of an amide, a sulfoxide or a amine oxide. Each polar group need not necessarily be the same as the other polar groups. In the most preferred embodiments, the poplar groups within the compound are the same as each other and the terminal polar groups are the same. Preferably, all the polar groups are amide groups attached to the compound at the nitrogen atom or at the carbon atom of the carbonyl radical. The amide group may comprise one or more hydrocarbon substituents, such as a lower alkyl or alkenyl groups, including branched or unbranched groups. The term "lower alkyl or alkenyl group" is intended to include saturated and unsaturated hydrocarbon groups with 1 to about 5 carbon atoms.

-17-

The embodiments where a and b are 0 and A is a carbonyl radical or a group having the structure:

$$\begin{array}{c} -N- \\ | \\ C=O \\ | \\ R_4 \end{array}$$

wherein R_4 is a hydrogen atom or a lower alkyl or alkenyl group, have proven to be most useful embodiments to date.

Particularly preferred are compounds where a and b are O, A is a carbonyl radical and R has the structure:

$$\begin{array}{c} R_2 \\ | \\ -N \\ | \\ R_3 \end{array}$$

wherein R_2 and R_3 each is hydrogen atom, a methyl group or a ethyl group.

The compound also requires at least two nonpolar sections, designated B and B_1, which are attached to and connect polar groups. Additional nonpolar sections may also be present, e.g. B_2 when a is 1 and B_3 when b is 1. The nonpolar sections may comprise linear saturated hydrocarbon chains, linear unsaturated hydrocarbon chains containing one or more double or triple bonds, or saturated or unsaturated hydrocarbon chains containing one or more lower alkyl or alkenyl groups or small carbocyclic rings as substituents. In one of the preferred embodiments, the nonpolar groups are hydrocarbon chains comprising 4 to 7 methylene groups, especially pr f rred ar hydrocarbon chains containing 6 carbon atoms.

-18-

Some of the preferred compounds for the practice of the present invention are those having the structures:

5

$$H-N-(CH_2)_6-N-(CH_2)_6-N-H$$

with $C=O$ groups below each N, each bearing CH_3

10

$$CH_3-N-C-(CH_2)_6-C-(CH_2)_6-C-N-CH_3$$

with carbonyl O above the C groups; $CH3$ below left N and CH_3 below right N

15

20

$$CH_3CH_2O$$

$$(CH3)_2-N-C-(CH2)_5-C-(CH2)_5-C-N-(CH3)_2$$

$$O \qquad OCH_2CH_3$$

25

30

35

$$(CH_3)_2-N-\overset{\overset{\displaystyle O}{\parallel}}{C}-(CH_2)_5-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle N-(CH_3)_2}{|}}{\underset{\underset{\displaystyle}{|}}{\overset{}{C}}}}-(CH_2)5-\overset{\overset{\displaystyle O}{\parallel}}{C}-N-(CH_3)_2$$

5

10

$$R-\overset{\overset{\displaystyle O}{\parallel}}{\underset{\underset{\displaystyle R}{|}}{N}}-C-(CH_2)_x-\overset{\overset{\displaystyle \underset{\displaystyle R}{\nwarrow}\underset{\displaystyle}{N}\nearrow \overset{\displaystyle R}{}}{\underset{\displaystyle C=O}{|}}}{CH}-(CH_2)_x-\overset{\overset{\displaystyle O}{\parallel}}{C}-\underset{\underset{\displaystyle R}{|}}{N}-R$$

15

20

$$R-\underset{\underset{\displaystyle R}{|}}{N}-\overset{\overset{\displaystyle O}{\parallel}}{C}-(CH_2)_x-\overset{\overset{\displaystyle O}{\parallel}}{C}-N-(CH_2)_x-\overset{\overset{\displaystyle O}{\parallel}}{C}-\underset{\underset{\displaystyle R}{|}}{N}-R$$
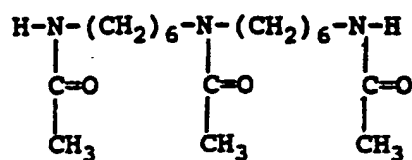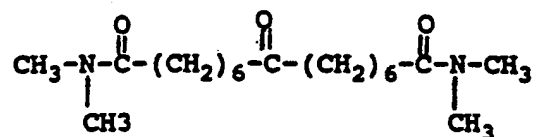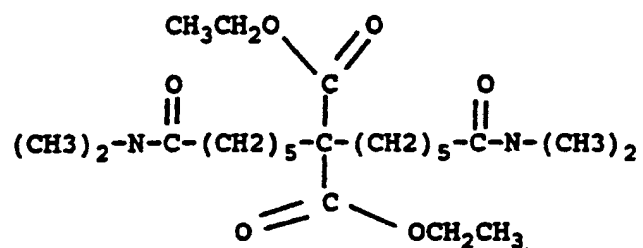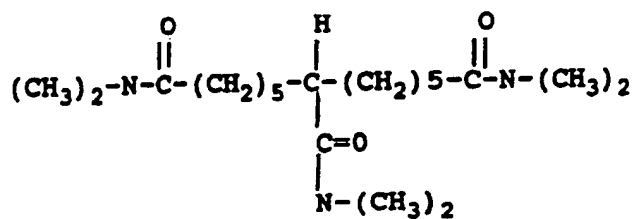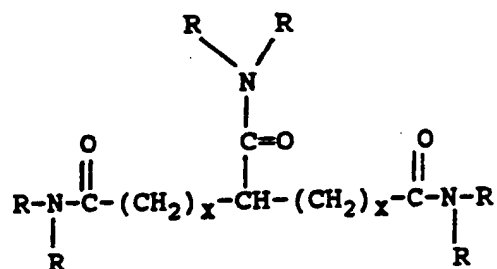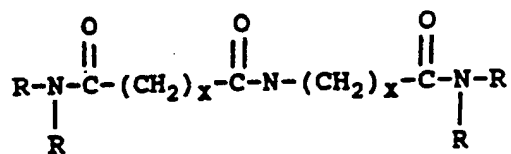
25

wherein R is hydrogen or a methyl group and x is 5 or 6.

30

35

Other compounds of this invention include a compound having the structure:

$$
\begin{array}{c}
\text{O} \\
\parallel \\
\text{CH}_3\text{-N} \qquad \text{N-CH}_3 \\
\text{O=} \qquad \text{=O} \\
(\text{CH}_3)_2\text{N-}\underset{\underset{\text{O}}{\parallel}}{\text{C}}\text{-(CH}_2)_5 \qquad (\text{CH}_2)_5\text{-}\underset{\underset{\text{O}}{\parallel}}{\text{C}}\text{-N(CH}_3)_2 \; ;
\end{array}
$$

or a compound having the structure:

$$
\begin{array}{c}
\text{O} \qquad\qquad \text{O} \\
\parallel \qquad\qquad \parallel \\
R_1\text{-O-C} \qquad\qquad \text{C-O-R}_2 \\
\text{C} \\
\text{H3C-N-}\underset{\underset{\text{O}}{\parallel}}{\text{C}}\text{-(CH}_2)_3 \qquad (\text{CH}_2)_3\text{-}\underset{\underset{\text{O}}{\parallel}}{\text{C}}\text{-N-CH}_3 \\
\underset{\text{H}_3\text{C}}{|} \qquad\qquad\qquad\qquad \underset{\text{CH}_3}{|}
\end{array}
$$

wherein R1 and R2 may be the same or different and each is a lower alkyl group;

Also provided by this invention is a compound having the structure:

$$
\begin{array}{c}
\text{O} \\
\parallel \\
\text{CH}_3\text{-N} \qquad \text{N-CH}_3 \\
\text{O=} \qquad \text{=O} \\
R \qquad (\text{CH}_2)\text{-}\underset{\underset{\text{O}}{\parallel}}{\text{C}}\text{-N-(CH}_3)_2
\end{array}
$$

wherein R is H or a lower alkyl group.

This invention further provides a compound having the structure:

5

$$CH_3-N-C-(CH_2)_n-C-N-CH_3$$

10

wherein n is an integer which is greater than 1 and R_1 and R_2 may be the same or different and each is H or a lower alkyl group.

15

A compound having the structure:

20

25

is also provided by this invention wherein X1 and X2 may independently be the same or different and each is N(CH3)2 NH-phenyl, or $HNCH_3$.

30

35

-22-

This invention also provides a compound having the
structure:

$$\begin{array}{ccc}
\text{H3C} & \text{O} & \text{O} & \text{CH3} \\
| & || & || & | \\
\text{OH-N-C-(CH}_2)_6\text{-C-N-OH}
\end{array}$$

or a compound having the structure:

$$\begin{array}{ccc}
\text{O} & \text{R1} & \text{R2} & \text{O} \\
|| & | & | & || \\
2((\text{CH3})2\text{-N-C})\text{-C-(CH2)n-C-(C-N-(CH3)2))2}
\end{array}$$

wherein R1 and R2 may be the same or different and each is
H or a lower alkyl group;

and n is an integer from 1 to 10.

This invention further provides a compound having the
structure:
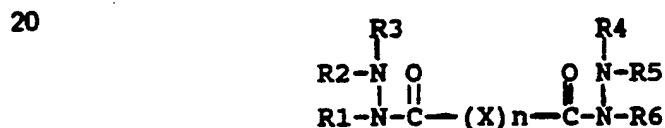
$$\begin{array}{cc}
\text{R3} & \text{R4} \\
| & | \\
\text{R2-N} \quad \text{O} & \text{O} \quad \text{N-R5} \\
| \quad || & || \quad | \\
\text{R1-N-C}\text{---}(\text{X})\text{n}\text{---}\text{C-N-R6}
\end{array}$$

wherein R1, R2, R3, F4, R5, R6 may independently be the same
or different from each other and is H or a lower alkyl
group; wherein X is methyl or phenyl; and n is an integer
from 1 to about 15.

A compound having the structure:

$$\begin{array}{c}
\text{R2-N} \text{---------} \text{N-R1} \\
| \qquad\qquad | \\
\text{O= C} \qquad\qquad \text{C=O} \\
\text{CH}_3 \quad \text{O} \qquad\qquad \text{C} \qquad\qquad \text{O} \quad \text{CH}_3 \\
| \quad || \qquad\qquad\qquad || \quad | \\
\text{CH}_3\text{-N-C-(CH2)}_5 \qquad (\text{CH2})5\text{-C-N-CH3}
\end{array}$$
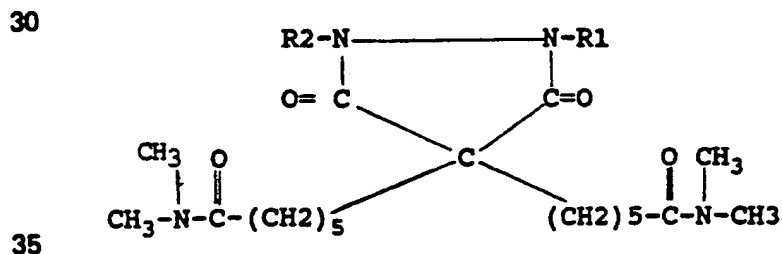
is also provided by this invention wherein R1 and R2 may be the same or different and is H or CH3.

5   The invention also provides a compound having the structure:
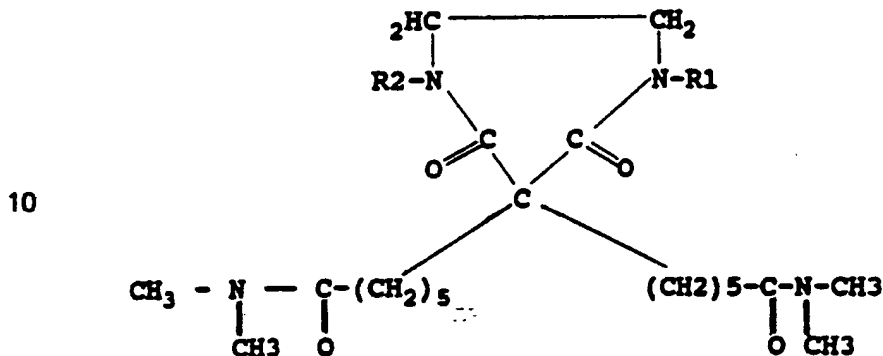
$$_2HC —— CH_2$$

R2-N   N-R1

10

$$CH_3 - N — C-(CH_2)_5 \qquad (CH2)5-C-N-CH3$$
$$CH3 \quad O \qquad\qquad O \quad CH3$$

wherein R_1 and R_2 may be the same or different and is H or

15   CH_3.

A compound having the structure:

$$HO-N - C - X - C - N - OH$$

with R_1, O, O, R_2 above the respective atoms

20
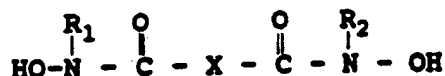
is provided by this invention, wherein X has the structure:

-CH=CH-⟨ ⟩-CH=CH-,   ⟨ ⟩-CH=CH- ,   ⟨ ⟩

25 -C≡C-⟨ ⟩-C≡C-,

⟨ ⟩-C≡C-,   -CH2-⟨ ⟩-CH2- ,   or

$$—CH_2 \qquad H$$
$$C = C$$
30    $$H \qquad CH_2·—$$

-CH=CH-CH=CH;   ⟨ ⟩-⟨ ⟩

35   wherein R^1 and R_2 are th same or different and is H or

lower alkyl group.

Further provided by this invention is a compound having the
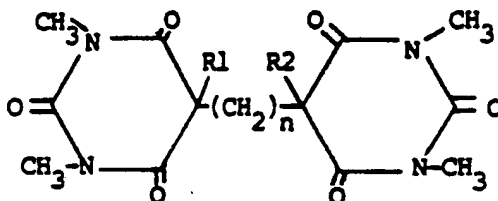structure:

5

$$(CH_3)N-C(=O)-...$$

wherein R1 and R1 may independently be the same or different
and each may be H or a lower alkyl group; wherein R3 and R4
may independently be the same or different and each may be
CH3 or OH; and wherein n is 5 or 6.

A compound having the structure:

20

$$(CH3)2-N-C(=O)-(CH2)6-C-(CH2)6-C(=O)-N-(CH3)2$$

is also provided by this invention, wherein R_1 and R_2 is the
same or different and is hydrogen, lower alkyl, alkenyl,
alkynyl, an amide or hydroxyamide.

This invention further provides compounds having the
following structures:

-N(CH3)-CO-(CH2)5-CO-N(CH3)-

35

-25-

and

H5C2-O   O-C2H5
    | |
  O=C   C=O
        \   /
          C
        /   \
HO-NH-CO-(CH2)5   (CH2)5-CO-NH-OH

also,

[benzene ring]—N(CH3)-CO—(CH2)5—CO-N(CH3)—[benzene ring]

and a compound having the structure:

C2H5   H
  | N—[benzene ring]
  O   /
  | C=O
  O=C   /
      \   /
2(CH3)N-CO-(CH2)5-C-(CH2)5-CO-N(CH3)2

The invention also concerns a method of selectively inducing terminal differentiation of neoplastic cells and thereby inhibiting proliferation of such cells which comprises contacting the cells under suitable conditions with an amount of the compounds shown above effective to selectively induce terminal differentiation in the cells.

A method of selectively inducing terminal differentiation of neoplastic cells and thereby inhibiting proliferation of such cells is further provided by this invention which comprises contacting the cells under suitable conditions with an amount of a compound effective to selectively induce terminal differentiation, the compound having the structure:

$$HOHN \overset{O}{\overset{\|}{C}} (X)_n \overset{O}{\overset{\|}{C}} NHOH$$
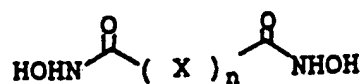
wherein X is phenyl or methyl and n is an int rg r from 1 to 15.

-26-

The contacting must be performed continuously for a
prolonged period of time, i.e. for at least 48 hours,
preferably for about 4-5 days or longer.

The method may be practiced in vivo or in vitro. If the
method is practiced in vitro, contacting may be effected by
incubating the cells with the compound. The concentration
of the compound in contact with the cells should be from
about 1 μM to about 25 mM, preferably from about 4 μM1 to
about 5 mM.

However, for the compound having the structure:

$$HO-NH-\underset{\underset{O}{\|}}{C}-(CH2)4—\underset{\underset{O}{\|}}{C}-NH-OH$$
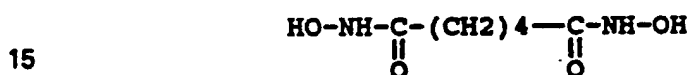
an amount of about 0.01 mM to about 10 mM has been shown to
be effective.

The concentration depends upon the individual compound. For
example, compound 12 of Table 1 should have a concentration
from about 0.1 to about 2 mM, preferably from about 0.5 to
about 0.7 mM. However, for the compounds listed in Table 4,
the optimal concentration is as low as 5 μM to about 5mM.
Another factor determining the preferable range is the state
of the tumor cells. Thus, in cells which have low levels of
vincristine resistance, the range of effective concentration
of compound 12 from Table 1 is from about 0.01 to about 0.3
mM with a preferable range of about 0.05 to about 0.1 mM.

The invention also concerns a method of treating a patient
having a tumor characterized by proliferation of neoplastic
cells which comprises administering to the patient an amount
of the compounds shown above, or with a compound having the
structure:

-27-

$$HOHN \overset{O}{\underset{}{\bigwedge}} (X)_n \overset{O}{\underset{}{\bigwedge}} NHOH$$
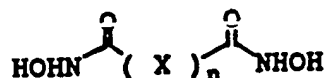
wherein X is phenyl or methyl and n is an integer from 1 to 15, effective to selectively induce terminal differentiation of such neoplastic cells thereby inhibiting their proliferation, and suppressing oncogenicity.

The method of the present invention is intended for the treatment of human patients with tumors. However, it is also likely that the method would be effective in the treatment of tumors in other animals. The term tumor is intended to include any cancer caused by the proliferation of neoplastic cells, such as lung cancer, acute lymphoid myeloma, bladder melanoma, renal carcinoma, breast carcinoma, or colorectal carcinoma. The administration of the compound to the patient may be effected orally or parenterally. To date, administration intravenously has proven to be effective. The administration of the compound must be performed continuously for a prolonged period of time, such as for at least 3 days preferably more than 5 days. In the most preferred embodiments, the administration is effected continuously for at least 10 days and is repeated at intervals wherein at each interval the administration is continuously effected for at least 10 days. For example, the administration may be effected at intervals as short as 5-10 days, up to about 25-35 days and continuously for at least 10 days during each such interval. The optimal interval period will vary depending on the type of patient and tumor. For example, in the incidence of acute leukemia, the so called myelodysplastic syndrome, continuous infusion would seem to be indicated so long as the patient tolerated the drug without toxicity and there was a positive respons .

Th amount of th compound administer d to the patient is

-28-

less than an amount which would cause toxicity in the
patient. In the certain embodiments, wherein the compound
has the structures:

5

$$(CH_3)_2-N-\underset{\underset{O}{\parallel}}{C}-(CH2)_5-\underset{\underset{\underset{C}{\parallel}}{\underset{O}{\parallel}}}{\overset{CH_3CH_2O}{\overset{\diagdown}{\underset{\diagup}{C}}}\overset{\diagup\!\!\!\diagup O}{}}-(CH2)_5-\underset{\underset{O}{\parallel}}{C}-N-(CH3)_2$$

10

15

$$(CH_3)_2-N-\underset{\underset{O}{\parallel}}{C}-(CH_2)_5-\underset{\underset{\underset{N-(CH_3)_2}{\mid}}{\overset{H}{\underset{\mid}{C}}}}{\overset{H}{\underset{C=O}{\mid}}}-(CH_2)5-\underset{\underset{O}{\parallel}}{C}-N-(CH_3)_2$$

20   or

$$\underset{\underset{\underset{CH_3}{\mid}}{\overset{C=O}{\mid}}}{H-N}-(CH_2)_6-\underset{\underset{\underset{CH_3}{\mid}}{\overset{C=O}{\mid}}}{N}-(CH_2)_6-\underset{\underset{\underset{CH_3}{\mid}}{\overset{C=O}{\mid}}}{N}-H$$
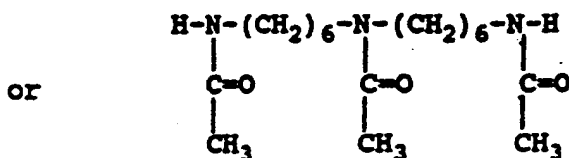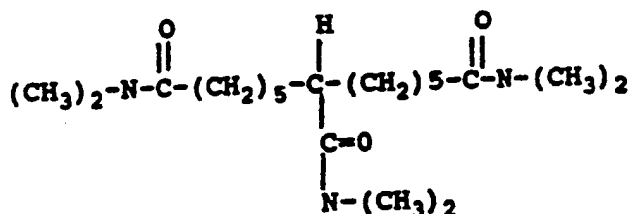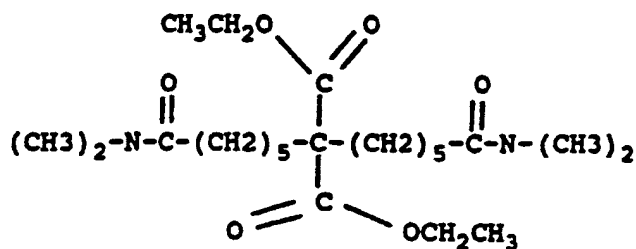
the amount of the compound which is administered to the
patient is less than the amount which causes a concentration
of the compound in the patient's plasma to equal or exceed
the toxic level of the compound.   Preferably,   the
concentration of the compound in the patient's plasma is
maintained at about 1.0 mM. It has been found with HMBA
that administration of the compound in an amount from about
5 gm/m^2/day to about 30 gm/m^2/day, particularly about 20
gm/m^2/day, is effective without producing toxicity in the
patient. For the compound shown above, in vitro studies
have shown that th optimal amount of th compounds is

25

30

35

substantially lower than 30 gm/m^2/day, and may even be lower than 1 gm/m^2/day. The optimal amount of the compound which should be administered to the patient in the practice of the present invention will depend on the particular compound used and the type of cancer being treated.

This invention, in addition to the above listed compounds, is intended to encompass the use of homologs and analogs of such compounds. In this context, homologs are molecules having substantial structural similarities to the above-described compounds and analogs are molecules having substantial biological similarities regardless of structural similarities.

The invention also concerns a pharmaceutical composition comprising a pharmaceutically acceptable carrier, such as sterile pyrogen-free water, and any of the compounds listed above in an amount less than an amount which if administered intravenously or orally to a patient would cause the patient's blood plasma concentration of the compound to exceed toxic levels.

The invention is illustrated in the Experimental Detail section which follows. This section is set forth to aid in an understanding of the invention but is not intended to, and should not be construed to, limit in any way the invention as set forth in the claims which follow thereafter.

-30-

Experimental Details
Methods
Cells and Materials

5   MELC 745A-DS19 cells and the variants of MELC derived from
this cell line, namely, the vincristine-resistant MELC V3.17
and VCR.C(2)15 cell lines (26), and the dimethylsulfoxide-
resistant cell line, DR10 (37), were maintained in alpha
minimal essential medium containing 10% fetal calf serum
10   (16). Cell cultures for all experiments were initiated with
cells in logarithmic growth phase (day 2 cultured cells) at
a density of 10^5 cells/ml. Inducer compounds were added in
the final concentrations indicated below, dissolved in
culture medium without fetal calf serum unless otherwise
15   indicated. Cell density and benzidine reactively were
determined as described (16).

Commitment to terminal differentiation, characterized by
limited cell division (colony size <32 cells) and
20   accumulation of hemoglobin (benzidine reactive colonies) was
assayed by a colony cloning assay using 2% methylcellulose
as described (25).

25

30

35

<u>Table 1</u>

CPD #	Structure	Mol Wt.	Optimal Concentration (mM)	Benzidine Reactive Cells (%)	Commitment (%)
1.	$CH_3-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-(CH_2)_6-\overset{H}{N}-\overset{O}{\overset{\|}{C}}-CH_3$	200	5	>95	>95
2.	$CH_3-\overset{H}{N}-\overset{O}{\overset{\|}{C}}-CH_3$	75	50	~70	NO
3.	$CH_3-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-(CH_2)_2-\overset{CH_3}{\underset{H}{C}}-(CH2)_2-\overset{H}{N}-\overset{O}{\overset{\|}{C}}-CH_3$	200	5	>95	>95
4.	$CH_3-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-(CH_2)_3-\overset{CH_3}{\underset{H}{C}}-CH_2-\overset{H}{N}-\overset{O}{\overset{\|}{C}}-CH_3$	200	5	>95	>90
5.	$CH_3-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-CH_2-\overset{H_3C}{\underset{H}{C}}-\overset{CH_3}{\underset{}{C}}-CH_2-\overset{H}{N}-\overset{O}{\overset{\|}{C}}-CH_3$	200	5	>95	NO
6.	$CH_3CH_2-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-(CH_2)_5-\overset{H}{N}-\overset{O}{\overset{\|}{C}}-CH_2CH_3$	218	5	>90	>90
7.	$CH_3-\overset{H}{N}-\overset{O}{\overset{\|}{C}}-(CH_2)_6-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-CH_3$	200	5	>95	>90
8.	$(CH_3)-N-\overset{O}{\overset{\|}{C}}-(CH_2)_6-\overset{O}{\overset{\|}{C}}-N-(CH_3)_2$	228	5	>90	>90
9.	$(CH_3)_2-N-\overset{O}{\overset{\|}{C}}-(CH_2)_7-\overset{O}{\overset{\|}{C}}-N-(CH_3)_2$	242	2	>90	>90
10.	$HN-(CH_2)_6-N-(CH_2)_6-NH$ with three $\overset{}{\underset{O}{C}}-CH_3$ groups	341	2	>90	>90
11.	$(CH_3)_2-N-\overset{O}{\overset{\|}{C}}-(CH_2)_5-\overset{H}{C}-(CH_2)_5-\overset{O}{\overset{\|}{C}}-N-(CH_3)_2$; $C=O$; $N-(CH_3)_7$	369	2.5	>90	>90
12.	$(CH_3)_2-N-\overset{O}{\overset{\|}{C}}-(CH_2)_5-C-(CH_2)_5-\overset{O}{\overset{\|}{C}}-N-(CH_3)_2$; $CH_3CH_2O-C{\overset{O}{\|}}$ above ; $O=C-OCH_2CH_3$ below	442	0.6	>90	>90

-32-

Table 2

		24h		48h		120h		Commitment	
		Cell count	B+	Cell count	B+	Cell count	B+	24h	48h
HMBA 1 mM	DS19	0.4	0-1	1.0	0-1	3.0 $\times 10.^6$	8%	0-1	6-8
	V$_3$17	0.38	8-9	0.8	33%	2.8	58%	30%	43%
2 mM	DS19	0.42	0-1	0.9	0-1	2.9	64%	0-1	71%
	V$_3$17	0.4	7-9	0.8	72%	2.6	89%	39%	79%
3 mM	DS19	0.4	0-1	0.9	2%	2.8	82%	0-1	81%
	V$_3$17	0.3	9-10	0.4	80%	1.8	91%	77%	94%
4 mM	DS19	0.3	0-1	0.8	11%	2.6	94%	4%	92%
	V$_3$17	0.28	9-13	0.4	88%	1.6	96%	80%	99%
5 mM	DS19	0.23	0-1	0.5	14%	2.4	98%	6%	96%
	V$_3$17	0.22	9-14	0.5	89%	1.6	99%	83%	98%
IC-135									
1 mM	DS19	0.3	0-1	0.6	0-1	1.6	69%	0-1	71%
	V$_3$17	0.3	8-9	0.6	83%	1.3	89%	58%	89%
2 mM	DS19	0.3	0-1	0.5	0-1	1.3	79%	1-2	87%
	V$_3$17	0.29	7-6	0.4	88%	1.3	94%	70%	95%
3 mM	DS19	0.27	0-1	0.4	0-1	1.3	86%	5-4	91%
	V$_3$17	0.12	8-6	0.3	89%	0.9	96%	76%	97%
4 mM	DS19	0.13	0-1	0.2	0-1	0.6	78%	2-1	79%
	V$_3$17	0.18	6-8	0.19	34%	0.5	79%	44%	81%
5 mM	DS19	0.12	0	0.13	0-1	0.4	--	8%	--
	V$_3$17	0.12	8-11	0.12	0-1	0.3	--	0-1	--
CONTROL		0.4	5-9	1.0	0.1	3.1 $\times 10^6$	0-1	0-1	0-1

IC-135 50 mM Stock 40 λ-- 1mM 200 λ-- 5mM
HMBA 200 mM Stock 10 λ-- 1mM 50 λ-- 5mM

-33-

TABLE 3
TESTING OF IC-135 ON DIFFERENT
MELC LINES

DS-1g

	1st Day	2nd Day	5th Day	5th B+	5 day Commit -ment
Control	0.38	1.1	2.8	0-1	0-1
5mM HMBA	0.2	0.5	2.0	91-90	88-88
0.5 mM IC-135	0.38	1	2.1	41-49	31-28
1mM IC-135	0.33	0.8	1.4	72-74	51-58
2mM IC-135	0.2	0.5	1.3	80-80	78-76
3mM IC-135	0.2	0.5	1.2	87-88	81-80

V3-17

	1st Day	2nd Day	5th Day	5th B+	5 day Commit -ment
Control	0.4	1.2	2.9	3-6	0-1
5mM HMBA	0.23	0.5	1.6	98-99	99-98
0.5 mM IC-135	0.4	0.6	2.7	40-49	36-34
1mM IC-135	0.31	0.44	1.3	89-93	89-91
2mM IC-135	0.21	0.4	1.0	96-91	92-99
3mM IC-135	0.23	0.3	0.9	98-97	99-99

DR-10

	1st Day	2nd Day	5th Day	5th B+	5 day Commit -ment
Control	0.41	1.0	2.9	0-1	0-0
5mM HMBA	0.3	0.48	2.1	90-89	1-0
0.5 mM IC-135	0.4	0.62	1.9	44-39	0-0
1mM IC-135	0.32	0.48	1.4	70-74	0-1
2mM IC-135	0.2	0.41	1.2	79-81	0-2
3mM IC-135	0.2	0.40	1.2	88-86	1-3

-34-

Chemistry

HMBA, compound 1 of Table 1, (9) was obtained from Aldrich
Chemical Co.

5

The preparation and characterization of compounds 1, 2, 6,
7, 8 and 9 of Table 1 (9,27) was previously described. All
new amides were purified by chromatography on alumina with
5% methanol in methylene chloride, and were judged pure by
10   thin layer chromatography (single spot) and ^{1}H NMR
spectroscopy. Final products were characterized by ^{1}H NMR,
infrared, and CI mass spectroscopy, while intermediates were
characterized by ^{1}H NMR spectra. The data were consistent
with the assigned structures (expected infrared and NMR
15   signals, M + 1 mass spectra).

For the synthesis of compound 3 (Table 1), the known 3-
methyl-1,5-dibromopentane (38) was converted to the bis-
phthalimide derivative, and this was cleaved with hydrazine
20   to afford 3-methyl-1,5-diaminopentane, isolated as the
dihydrochloride (m.p. 123-126°). This was converted to
compound 3 with acetic anhydride and triethylamine in
dioxane.

25   Compound 4 (Table 1) (m.p. 67-68°) was obtained in
quantitative yield by similar acetylation of commerically
available 2-methyl-1,5-diaminopentane.

Compound 5 (Table 1) was synthesized from meso-2,3-
30   dimethylsuccinic acid in six steps. Reduction of the
dimethyl ester with LiAlH$_4$ afforded the meso-2,3-
dimethylbutanediol (92% yield). This was converted to the
bis-tosylat , and then to the bis-phtalimide. Deprotection
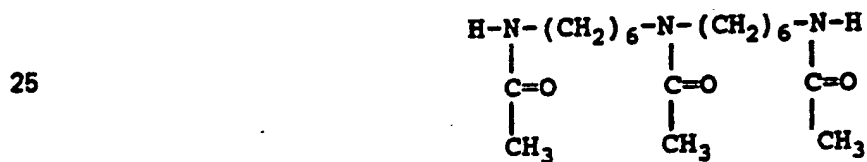and acetylation as befor gave compound 5 as an oil (61%

35

-35-

Compound 10 (Table 1) was prepared by making a solution of 19.8gm commercial bis-hexamethylenetriamine in 500 ml of 1,4-dioxane at room temperature under argon. Then 44.8 ml. of triethylamine was added, and 20.3 ml. of acetyl chloride was slowly added with stirring. After two hours of stirring at room temperature the triethylamine hydrochloride was removed by filtration and the filtrate was concentrated in vacuo. The product triacetyl compound was isolated as a clear viscous oil at about 90% yield by chromatography on basic alumina using isopropanol/ethyl acetate/dichloromethane in the ratio 2/3/5. On thin layer plates of basic alumina with this solvent mixture the product had an R_f of ca. 0.6.

The mass spectrum (chemical ionization, NH_3 carrier) showed peaks at 342 (100%, M + 1), 227 (10%) and 115 (22%). The infrared spectrum (thin film on NaCl) had bands at 3288, 2931, 2858, 1627, 1560, 1437, 1373, and 1292 cm^{-1}. In the proton NMR ($CDCl_3$) the acetyl groups appeared at 6.10 as a broad signal, while the methylene protons appeared as multiples with the expected intensities in the regions of 3.12 to 3.30 and 1.21 to 1.54.

$$H-N-(CH_2)_6-N-(CH_2)_6-N-H$$
$$\quad | \qquad\qquad | \qquad\qquad |$$
$$\quad C=O \qquad\quad C=O \qquad\quad C=O$$
$$\quad | \qquad\qquad | \qquad\qquad |$$
$$\quad CH_3 \qquad\quad CH_3 \qquad\quad CH_3$$

bis-Hexamethylenetriacetamide (IC-135)

-36-

For preparation of the triamide compound 11 (Table 1),
dimethyl malonate was dialkylated with ethyl 6-
bromohexanoate under standard conditions. The resulting
tetraester was then hydrolyzed and thermally
monodecarboxylated to 1,7,13-tridecanetricarboxylic acid.
Treatment with thionyl chloride followed by diethylamine
afforded compound 11 (Table 1).

Compound 12 (Table 1) was prepared by simple dialkylation of
dimethyl malonate with the know N,N'-dimethyl-6-
bromohexanecarboxamide (39).

The compound having the structure (Compound 25, Table IV):
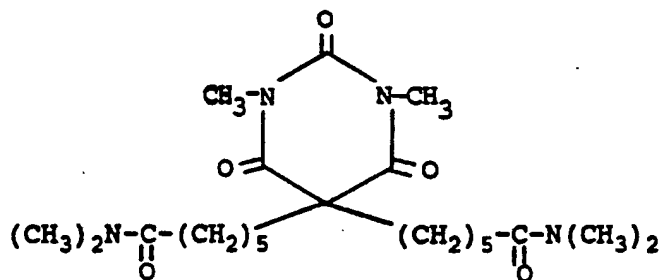
was made as follows. 1,3-dimethylbarbituric acid (5g; 0.032
mol) in DMF (100 ml) was slowly added to a suspension of
sodium hydride (1.5g; 0.064 mol) in DMF (300 ml). The
suspension was stirred at 80°C for five hours. N,N-Dimethyl
6-bromohexanoylamide (14.3 g; 0.064 mol) in DMF (100 ml) was
added to the cool suspension with stirring. The resulting
suspension was stirred at 120°C overnight. The DMF was
vaporated and the residue was partitioned between
chloroform and water (100-100 ml). The chloroform layer was
separat d, and the water layer was extracted with chloroform

(5 x 50 ml). The combined chloroform layers were dried over anhydrous magnesium sulfate, and evaporated. The oily residue was purified by column chromatography on silica gel in ethyl acetate-tetrahydrofuran (2:1). The yield of 1,3-dimethyl-5,5-di(N,N-dimethyl-5-pentylcaboxamide)barbituric acid was 4.1 g (30%).

^{1}H NMR (CDCl$_3$, 200 MHz) δ3.33(s, bar, N-CH3,6H), 2.98 (s, N-CH3, 6H), 2.93 (s, N-CH3, 6H), 2.24 (t, J=7 Hz, CH2CON, 4H), 1.96 (m, 4H), 1.58 (m, 4H), 1.28 (m, 4H), 1.08 (m, 4H).

The compounds having the structure (Compound 29, Table IV):
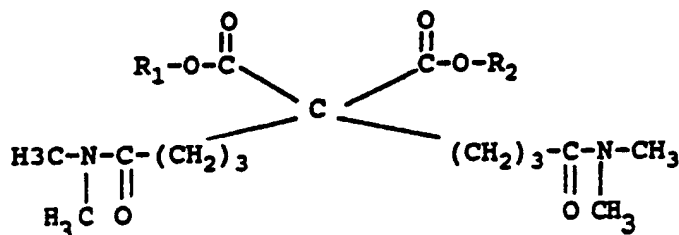
wherein R_1 and R_2 may be the same or different and each is a lower alkyl group and n is an integer greater than 1,was made as follows. Diethyl malonate (50.6 ml; 53.3 g; 0.33 mol) in DMF (150 ml) was added slowly to a cool suspension of sodium hydride (16 g; 0.67 mol) in DMF (1 liter). The suspension was carefully heated with stirring at 80° C (about one hour). The clear mixture was cooled, and N,N-dimethyl 6-bromohexanoylamide (146.5 g; 0.66 mol) in DMF (200 ml) was slowly added at room temperature. The suspension was stirred at 110°C for two hours, and the DMF was evaporated. The semisolid residue was partitioned between chloroform and water (about 200-200 ml). The organic layer was separated, and the water layer was xtracted with chloroform (5 x 100 ml). The combined chloroform layers wer dri d over anhydrous magn sium sulfated and evaporated. The oily residue was purified by

-38-

column chromatography on silica gel in ethyl acetate-
tetrahydrofuran (4:1). The yield of diethyl undecane-6,6-
di(ethylcarboxylate)-1,11-di(N,N-dimethlycarboxamide) was
106 g (76%).

5

^{1}H-NMR(CDCl3, 200 MHz) δ4.11(q, J=7.0 Hz, 4H), 2.96
(t,J=7.5Hz, 1H), 2.89(s,6H), 2.25(t, J=7.2Hz, 4H), 1.88 (m,
4H), 1.59 (m, 4H), 1.16-1.31 (m,14H).

10

IR (NaCl, CH2Cl2) 2937, 2866, 1728, 1642, 1498, 1462, 1399,
1369, 1265, 1153, 1097, 1029 cm^{-1}.

MS (FAB, glycerol) m/e=443 (40%, M+1$^+$), 398 (25%), 295
(80%), 142 (45%).

15

The compound having the structure:

20
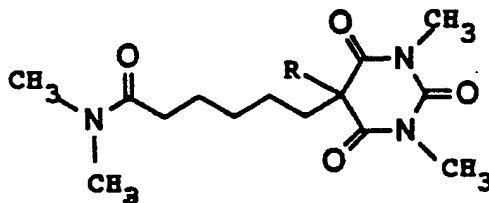
was made as follows. 1,3,5,-trimethylbarbituric acid (1.7g;
25   10 mmol) in DMF (20ml) was added at room temperature to a
suspension of sodium hydride (0.24 g; 10 mmol) in DMF (100
ml). The suspension was stirred at 90°C for one hour. N,N-
Dimethyl 6-bromohexanoylamide (2.22 g; 10 mmol) in DMF (20
ml) was added to a cool mixture (about 5°C). The suspension
30   was stirred at 110°C overnight. DMF was evaporated, and the
residue was partitioned between chloroform and water (50-50
ml). The chloroform layer was separated, and the water
layer was extracted with chlorof rm. The combined
chloroform layers were dried over anhydrous magnesium
35   sulfate and evaporated. The oily residue was purified by

-39-

column chromatography on silica gel in ethyl acetate-tetrahydrofuran. The yield of 1,3-dimethyl-5-methyl-5-(5-pentyl-N,N-dimethylcarboxamide)barbituric acid was 1.5 g(48%).

^{1}H NMR (CDCl$_3$, 200 MHz) δ3.23(s, bar.H, N-CH3,6H), 2.99 (s, N-CH3, 3H), 2.94 (s, N-CH3, 3H), 2.26 (t, J=7.2 Hz, CH2CON, 2H), 1.98 (m, 2H), 1.62 (m, 2H), 1.52 (m, C-Me, 3H), 1.32 (m, 2H), 1.11 (m, 2H).

The compounds having the structure:
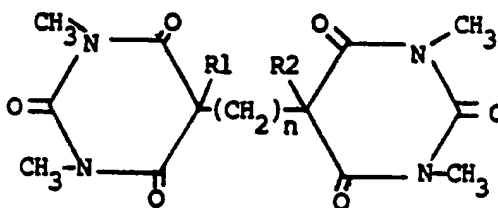
were made as follows. 1,3,5-trimethylbarbituric acid (0.71 g; 0.029 mol)in DMF (10 ml) was added to a suspension of sodium hydride (0.71g; 0.029 mol) in DMF (20 ml). The suspension was stirred at 80°C for one hour. 1,n-dibromoalkane (0.0147 mol) was added to a cool suspension (about 5°C). The reaction mixture was heated at 110° C for four hours, cooled down and evaporated. The solid residue was slurried in water (about 200 ml), filtered, and the white solid was washed with water (3 x 5 ml), ethanol (3 x 20 ml), and ether (2 x 20 ml). The yield of dibarbiruric acids were for: n=3, 75%; n=4, 78%; n=5, 71%; n=6, 79%; n=7, 83%.

^{1}H NMR for the compound with n=2 (CDCl$_3$, 200 MHz) δ3.32(s, N-CH3, 12H), 1.86 (s,CH2,4H), 1.47 (s, C-CH3,6H).

-40-

The compounds having the structure:

```
           X1                    X2
           |                     |
        O=C                      C=O
            \                   /
             \                 /
              \               /
               C
              / \
             /   \
    CH3     /     \           CH3
     |     /       \           |
CH3—N-C-(CH2)5   (CH2)5-C-N-CH3
     ||                       ||
     O                        O
```
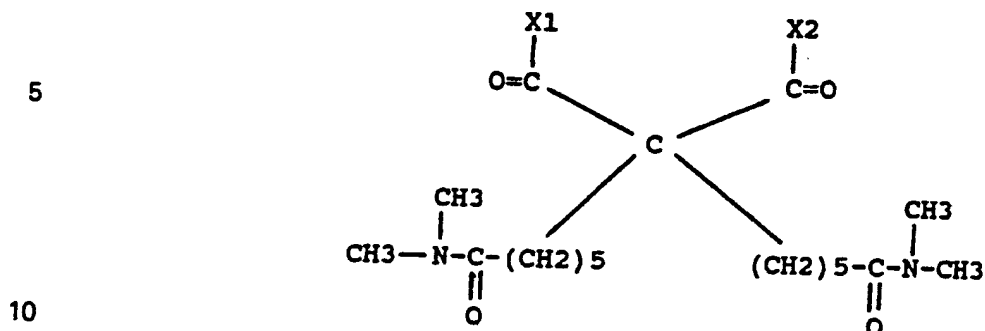
were made as follows. When X1 and X2 are the same and each is $HNCH_3$, aqueous (10 ml) potassium hydroxide (1.53 g; 27.5 mmol) was added to the diamidebarbituric acid (2.4 g; 5.5 mmol) in water (50 ml). The mixture was stirred at 70° C for fifteen minutes, cooled, filtered, acidified, and extracted by chloroform (5 x 20 ml). The combined chloroform extracts were dried over anhydrous magnesium sulfate and evaporated to the oily residue. By the NMR, hexamethylamide was more than 96% pure. The yield of undecane-1,11-di-(N,N-dimethylcarboxamide)-6,6-di(N-methylcarboxamide) was 2.1 g (93%).

^1H NMR ($CDCl_3$, 200 MHz) $\delta 7.38$ (q, J=8 Hz), 3.03 (s, N-CH3,6H), 2.94 (s,N-CH3,6H), 2.8 (d, J=8 Hz, N-CH3,6H), 2.32 (t, J=7.2 Hz, CH2CON, 4H), 2.82 (m, 4H), 2.62 (m, 4H), 1.25 (m, 8H).

When X1 and X2 are the same and each is N(CH3)2, the hexamethylamide (2 g; 4.9 mol) in DMF (10 ml) was added at room temperature to a suspension of sodium hydride (0.24 g; 0.01 mol) in DMF (20 ml). The mixture was stirred at 80°C for one hour. Methyl iodide (3.5 ml; 8 g; 56 mmol) was added with stirring to the cool reaction mixture. The r action was continued at 60°C overnight. The solvent was evaporated and the solid residue was dissolved in water (50
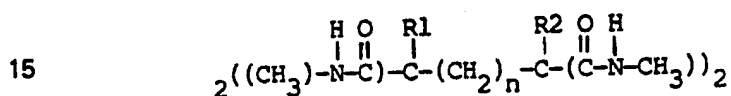
ml) and the water solution was extracted with 8:2 chloroform-methanol (5 x 50 ml). The combined organic layers were dissolved in acetone (200 ml) and filtered through a short column of silica gel. Acetone was evaporated yielding pure undecane-1,6,6,11-tetra-(N,N-dimethylcarboxamide) (1.36 g; 63%).

^{1}H NMR (CDCl$_3$) δ2.99 (s, N-CH3, 6H), 2.94 (s, N-CH3, 6H), 2.92 (s, N-CH3, 6H), 2.88 (s, N-CH3, 6H), 2.28 (t, J=7.2 Hz, CH2CON, 4H), 1.86 (m, 4H), 1.62 (m, 4H), 1.32 (m, 8H).

The compounds having the structure:

$$_2((CH_3)-N-\overset{\overset{H}{|}\,\overset{O}{\|}}{C})-\overset{\overset{R1}{|}}{C}-(CH_2)_n-\overset{\overset{R2}{|}}{C}-(\overset{\overset{O}{\|}}{C}-N-CH_3))_2$$

wherein R$_1$ and R$_2$ may be the same or different and each is H or a lower alkyl group and wherein n is an integer from 1 to 15 are made as follows. A dibarbituric acid (3.66 g; 0.01 mol for n = 2) was suspended in 9:1 water-dioxane (60 ml) and heated up to 100°C. To this suspension was added at 110°C a solution of potassium hydroxide (5.6 g; 0.1 mol/20 ml water for n=2). The suspension immediately became a solution. The solution was heated at 110°C for an extra 10 minutes, cooled down, filtered, and acidified. The clear water solution was extracted with chloroform (10 x 50 ml). The combined chloroform extracts were dried over anhydrous magnesium sulfate, and evaporated. The yields of the alkane-1,1,n,n,-tetra-(N-methylcarboxamide) were for: n=2, 85%; n=3, 79%; n=4, 83%; n=5, 81%; n=6, 74%; n=7, 83%.
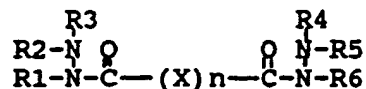
^{1}H NMR (CDCl$_3$, 200 MHz) for compounds having n=2, δ7.92 (q, J=4.6 Hz, NH-Me, 4H), 2.85 (d, J=4.6 Hz, N-CH3, 12H), 1.85

-42-

(s, CH2, 4H), 1.43 (s, C-CH3, 6H).

The compounds having the structure:

$$
\begin{array}{ccc}
\text{R3} & & \text{R4} \\
\text{R2-N} & \text{O} & \text{O} & \text{N-R5} \\
\text{R1-N-C} & \text{(X)n} & \text{C-N-R6}
\end{array}
$$

wherein R1, R2, R3, R4, R5, R6 may independently be the same or different from each other and is H or a lower alkyl group; wherein X is methyl or phenyl; and n is an integer from 1 to about 15, were made as follows. When R1 and R6 is each H and R2, R3, R4, and R5 is each CH3, to a chloroform (200 ml) solution of suberoyl chloride (8 g; 38 mmol) was added at room temperature to 1,1-dimethylhydrazine (11.5 ml; 9.1 g; 152 mmol). The mixture was stirred at room temperature for about three hours. The solvent was evaporated, and the residue was dissolved in methanol (400 ml). The methanol was evaporated and the solid residue was dissolved in water (100 ml). The water solution was extracted with hexanes (5 x 50 ml), and with chloroform (5 x 50 ml). The combined extracts were dried over anhydrous magnesium sulfate and evaporated. The solid residue was crystallized from acetone. The yield of hexane-1,6-di(N^2,N^2-dimethyl-carboxhydrazide) was 6.5 g (66%).

When R1, R2, R3, R4, R5, and R6 is each CH3, to a suspension
of sodium hydride (1.4 g; 57.9 mmol) in DMF (60 ml) at room
temperature was added tetramethylhydrazide (5 g; 19.3 mmol)
followed by methyl iodide (31.5 ml; 71.8 g; 579 mmol). The
reaction mixture was stirred at 60°C for five hours. The
DMF was evaporated, and the solid residue was dissolved in
water (50 ml) and extracted with chloroform (5 x 50 ml).
The chloroform extracts were dried over anhydrous magnesium
sulfate and evaporated.   The product was purified by
crystallization from hexanes.   The yield of hexane-1,6-
di(N^1,N^2,2-trimethylcarboxhydrazide) was 3.7 g (67 %).
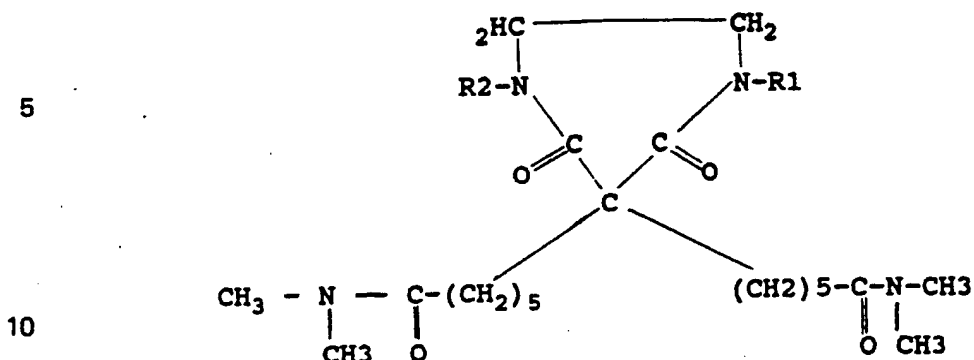
The compounds having the structure:
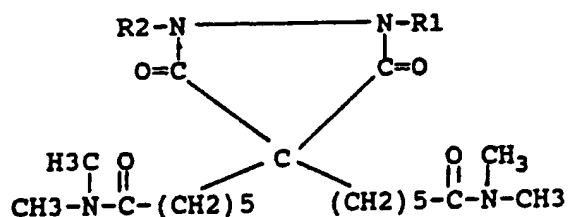
may be made as follows. The heterocyclic compounds may be
alkylated after they are formed from malonic chloride and
N,N^1-dialkylahydrazine.

-44-

The compounds having the structure:

$$
\begin{array}{c}
_2HC \underline{\hspace{3cm}} CH_2 \\
R2-N \hspace{2.5cm} N-R1 \\
C \hspace{0.5cm} C \\
O \hspace{2cm} O \\
C \\
CH_3 - N - C-(CH_2)_5 \hspace{2cm} (CH2)5-C-N-CH3 \\
CH3 \hspace{0.3cm} O \hspace{3cm} O \hspace{0.2cm} CH3
\end{array}
$$

wherein R_1 and R_2 may be the same or different and is H or CH_3 may be made as follows. The heterocyclic part of the compound may be made from malonic chloride and N,N^1-dealkylethylenediamine. The heterocyclic compound may then be alkylated with the corresponding alkyl bromide under known conditions.

The compounds having the structure:

$$HO-NH-CO-C\equiv C \underline{\hspace{2cm}} C\equiv C-CO-NH-OH$$

and

$$HO-NH-CO \underline{\hspace{2cm}} C\equiv C-CO-NH-OH$$
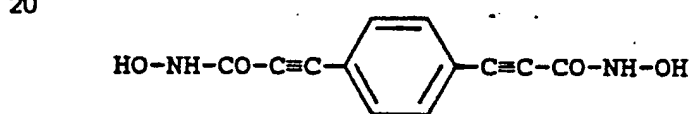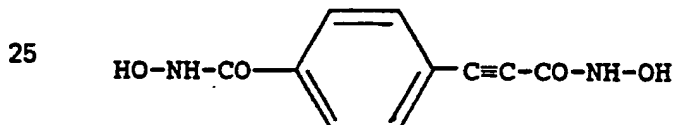
may be synthesized from the corresponding acid via acidic chloride with hydroxylamine hydrochloride in a solution of tetrahydrofuran and water. The acid may be made from 1,4-benzenediacrylic acid by an addition of bromine, and following elimination with base.

-45-

The compound having the structure (Compound 30, Table IV):

5

was made as follows. A suspension of 4-carboxycinnamic acid
(10 g; 0.052 mol) and thionyl chloride (15.2 ml), 24.8 g;
0.208 mol) in benzene (500 ml) was refluxed for five hours.
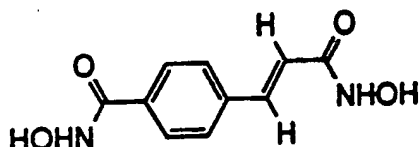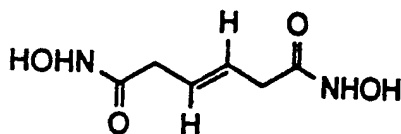The solvent was evaporated and the oily residue was
dissolved in tetrahydrofuran (100 ml). The tetrahydrofuran
solution was slowly added at about 5°C to a stirred water
solution (100 ml) of hydroxylamine hydrochloride (7.3 g;
0.104 mol) and potassium hydroxide (11.6 g; 0.208 mol). A
white precipitate was formed immediately. The suspension
was stirred at room temperature for two hours, and filtered.
The solid was washed with cooled water (5 x 20 ml) and
acetone (5 x 20 ml). The solid was slurried in methanol (3
liters), and the suspension was refluxed. The hot
suspension was filtered. The filtrate was concentrated to
100 ml and cooled. The white crystals were separated by
filtration, and washed with acetone (3 x 20 ml) and ether (5
x 20 ml). The yield of benzene-1-(N-hydroxycarboxamide)-4-
[trans-ethene-2-(N-hydroxycarboxamide)] was 5.2 g (45%).

^{1}H NMR (DMSO-d$_6$, 200 MHz), 11.05 (broad s, OH, 2H), 9.12
(broad s, NH, 2H), 7.77 (d, J=8.2 Hz, arom. H, 2H), 7.62 (d,
J=8.2 Hz, arom. H, 2H), 7.47 (d, J=15.8 Hz, CH, 2H), 6.53
(d, J=15.8 Hz, CH, 2H).

-46-

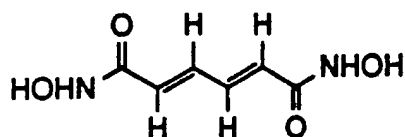The compound having the structure:

5

10

was made as follows. A suspension of trans-B-hydromuconic
acid (5 g; 0.0347 mol), oxalyl chloride (12 ml; 17.6 g;
0.139 mol) in benzene (250 ml) and a few drops of DMF was
stirred at room temperature until the reaction suspension
became clear (about one hour). The solvent was evaporated,
and the oily residue was dissolved in tetrahydrofuran (50
ml). The tetrahydrofuran solution was slowly added into
cool (about 5°C) aqueous solution of hydroxylamine
hydrochloride (4.8 g; 0.0694 mol) and potassium hydroxide
(7.8 g; 0.1388 mol). The reaction suspension was stirred at
room temperature for about one hour and evaporated to a
volume of 20 ml. The suspension was cooled and filtered.
The crude solid product was slurried in methanol (200 ml)
and the suspension was cooled and filtered. The filtrate
was dried over anhydrous magnesium sulfate, and evaporated
to a solid residue. The solid was slurried in ether (250
ml) and recovered by filtration. The yield of trans-2-
butene-1,4-di-(N-hydroxylcarboxamide) was 3.8 g (63%).

^{1}H NMR (DMSO-d_6, 200 MHz), δ 10.85 (s, OH, 2H), 9.08 (s, NH,
2H), 7.11 (d of d, J^1=11.2 Hz, J^2=2.8 Hz, CH, 2H), 6.17 (d
of d, J^1=11.4 Hz, J^2=2.8 Hz).

15

20

25

30

35

-47-

The compound having the structure:

5

HOHN—C(=O)—CH=CH—CH=CH—C(=O)—NHOH

(structure with O, H, H labels above and H, H, O labels below)

10

was made as follows. A solution of trans, trans-muconic
acid (5 g; 0.035 mol), oxalyl chloride (12.3 ml, 17.9 g;
0.141 mol) and a few drops N,N-dimethyl formamide in benzene
(250 ml) was stirred at room temperature for three hours.
The solvent was removed under reduced pressure. The oily
residue was dissolved in tetrahydrofuran (50 ml) and the
tetrahydrofuran solution was added dropwise to the stirred
solution of hydroxylamine hydrochloride (4.9 g; 0.074 mol)
and potassium hydroxide (7.9 g; 0.141 mol) at about 5°C. A
white precipitate was formed immediately. The suspension
was stirred at room temperature for about one hour and
filtered. The solid was slurried in methanol (2 liters) and
the suspension then was boiled and filtered. The methanol
solution was concentrated to a volume of about 20 ml. The
solid product was separated by filtration and washed with
ether (5 x 20 ml). The yield of trans,trans-1,3-butadiene-
1,4-(N-hydroxylcarboxamide) was 3.1 g (52%).

-48-

¹H NMR (DMSO-d₆, 200 MHz), 10.46 (s, 2H, OH), 8.75 (s, 2H, NH), 5.53 (t, J=3.4 Hz, CH, 2H), 2.70 (d, J=3.4 Hz, 4H, CH2).

The compound having the structure (Compound 24, Table IV):
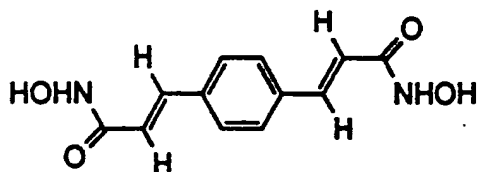
was made as follows. A suspension of 1,4-phenylenediacrylic acid (10 g; 0.184 mol) and thionyl chloride (13.4 ml; 21.9 g; 0.184 mol) in benzene (500 ml) was refluxed for five hours. The clear solution was cooled, and the solvent was evaporated. The oily residue was dissolved in tetrahydrofuran (100 ml) and slowly added into cooled (about 5°C) aqueous (100 ml) solution of hydroxylamine hydrochloride (6.4 g; 0.092 mol) and potassium hydroxide (10.3 g; 0.184 mol). A white precipitate was formed immediately. The suspension was stirred at room temperature (two hours), and filtered. The crude product was slurried in boiling water (100 ml), and the suspension was filtered. The solid was slurried in acetone (200 ml) and boiled. The hot suspension was filtered, and the solid was washed with acetone (5 x 20 ml), and th n with ether (5 x 20 ml). The yield of benzene-1,4-di[trans-ethene-2-(N-hydroxulcarboxamide)] was 6.2 g (54%).

^{1}H NMR (DMSO-d$_6$, 200 Hz), δ 10.82 (s, OH, 2H), 9.17 (s, NH, 2H), 7.58 (s, C$_6$H$_4$, 4H), 7.45 (d, J=15.8 Hz, CH, 4H), 6.51 (d, J=15.8 Hz).

The compound having the structure:
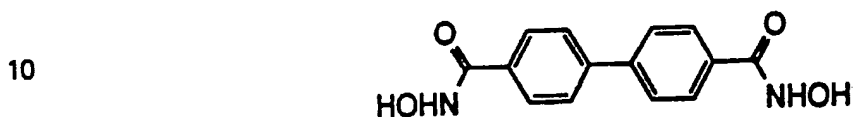
was made as follows. A suspension of 4,4-diphenyldicar-boxylic acid (5 g; 20.6 mmol) and thionyl chloride (7.3 ml; 12 g; 0.1 mmol) in benzene (250 ml) was refluxed overnight. The clear solution was cooled, and the solvent was evaporated. The solid residue was dissolved in tetrahydrofuran (100 ml) and was added slowly into a cool (about 5°C) aqueous (100 ml) solution of hydroxylamine hydrochloride (2.9 g; 41.7 mmol) and potassium hydroxide (4.65 g; 83 mmol). A white precipitate was formed immediately. The suspension was stirred at room temperature for about two hours and filtered. The crude product was slurried in water (50 ml) and the resulting suspension was boiled (about 10 minutes). The solid was separated by filtration, and slurried in acetone (200 ml) and boiled. The hot suspension was filtered, and the solid was washed with acetone (5 x 50 ml), and then with ether (5 x 20 ml). The yield of diphenyl-4,4'-di-(N-hydroxylcarbox-amide) was 4.9 g (88%).

^{1}H NMR (DMSO-d$_6$, 200 MHz), δ 11.33 (s, OH, 2H), 9.12 (broad s, NH, 2H), 7.87 (d, J=8.2 Hz, arom. H, 2H), 7.80 (d, J=8.4 Hz, arom. H, 2H).

-50-

The compound having the structure:

5

$$H5C2-O \qquad\qquad O-C2H5$$
$$O=C \qquad\qquad\qquad C=O$$
$$C$$
$$HO-NH-CO-(CH2)5 \qquad\qquad (CH2)5-CO-NH-OH$$
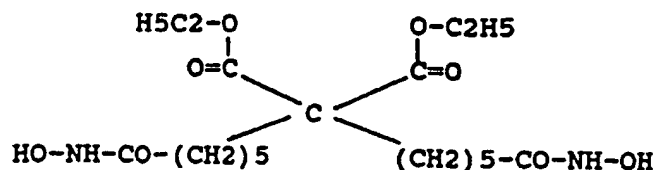
10   was made as follows. Diethyl malonate (15.2 ml; 16 g; 0.1
        mol) was slowly added at room temperature to a suspension of
        sodium hydride (4.8 g; 0.2 mol) in N,N-dimethyl formamide
        (300 ml). The suspension was heated at 80°C for about one
        hour and cooled down. Benzyl-6-bromohexanoate (57 g; 0.2
15   mol) was added at room temperature with vigorous stirring.
        The suspension was heated at 100°C for about 5 hours and the
        DMF was evaporated. The residue was partitioned between
        chloroform and water (200-200 ml). The chloroform layer was
        separated and the water layer was extracted with chloroform
20   (5 x 50 ml). The combined chloroform layers were dried over
        anhydrous magnesium sulfate, and evaporated. The oily
        residue was purified by column chromatography on silica gel
        in hexanes-ethyl acetate. The yield of undecane-6,6-
        di(ethylcarboxylate)-1,11-di(benzylcarboxylate) was 41 g (72
25   %). Dibenzyl 6,6-di(ethoxycarbonyl)-1,11-undecanedicar-
        boxylate (20 g; 35.2 mmol) was dissolved in methanol (150
        ml) and 5 % Pd-C (200 mg) was added. The reaction
        suspension was hydrogenated at room temperature overnight.
        The catalyst was removed by filtration, and the methanol was
30   evaporated yielding pure 6,6-(ethylcarboxylate)-1,11-
        undecanedicarboxylic acid (12.5 g; 93%).

        The acid was suspended in benzene (500 ml), and oxalyl
        chloride (11.2 ml; 16.3 g; 0.129 mol) and a few drops of DMF
35   were added. The mixture was stirred at room temperature for

about 3 hours. The solvent was evaporated and the oily residue was dissolved in chloroform (100 ml). The chloroform solution of the acid chloride was slowly added to a stirred solution of O-benzylhydroxylamine (16.3 g; 0.132 mol) in chloroform (300 ml). A white precipitate was formed immediately. The suspension was filtered, and concentrated hydrochloric acid was added (50 ml) to the chloroform filtrate and again filtered. The chloroform layer was washed with concentrated hydrochloric acid (3 x 100 ml), water (3 x 100 ml), and dried over anhydrous magnesium sulfate. The chloroform was evaporated yielding pure N,N'-dibenzyloxy-6,6-di(ethoxycarbonyl)-1,11-undecanedicarboxamide (17.1 g; 89%).

The benzyloxyamide (6 g; 0.01 mol) was dissolved in methanol (50 ml), and 5 % Pd-C (200 mg) was added. The methanol suspension was hydrogenated at room temperature overnight. The catalyst was separated by filtration and methanol was evaporated yielding pure undecane-6,6-di(ethylcarboxylate)-1,11-di(N-hydroxylcarboxamide) (3.9 g; 93%). The overall yield was 55.4%.

^{1}H NMR (DMSO-d_6, 200 MHz), 10.30 (broad s, OH, 2H), 8.75 (broad s, NH, 2H), 4.10 (q, J=7 Hz, COOCH2CH3, 4H), 1.90 (t, J=7.2 Hz, CH2CONHOH, 4H), 1.71 (m, 4H), 1.45 (m, 4H), 1.18 (m, 8H), 1.14 (t, J=7 Hz, COOCH2CH3, 6H).

The compounds having the structure (Compound 24, Table IV):
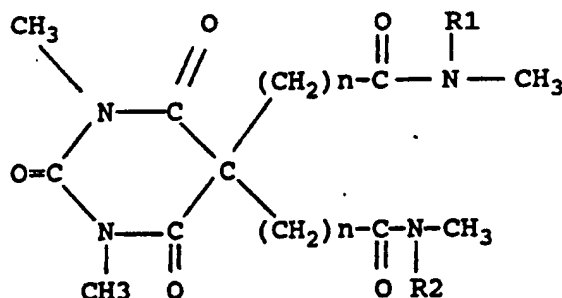
-52-

wherein R1 and R1 may independently be the same or different and is H or a lower alkyl group, wherein R3 and R4 may independently be the same or different and each is CH3 or OH, and wherein n is 5 or 6 were made as follows. 1,3-dimethylbarbituric acid (15.6 g; 0.1 mol) was slowly added at room temperature to a suspension of sodium hydride (4.8 g; 0.2 mol) in N,N-dimethylformamide (300 ml). The reaction was heated with stirring at 100°C for about three hours. A DMF (200 ml) solution of benzyl 6-bromohexanoate (57 g; 0.2 mol) was slowly added to the cool suspension with vigorous stirring. The suspension was heated at 110°C overnight, and the solvent was evaporated. The residue was partitioned between chloroform and water (300-300 ml). The chloroform layer was separated and the water layer was extracted with chloroform (5 x 50 ml). The combined chloroform layers were dried over anhydrous magnesium sulfate, and evaporated. The oily residue was purified by column chromatography on silica gel in ethyl acetate-tetrahydrofuran (9:1). The yield of dibenzyl ester was 18 g (32%).

The dibenzyl ester (18 g; 0.032 mol) was dissolved in methanol (50 ml) and catalyst (200mg of 5 % Pd-C) was added. The suspension was hydrogenated overnight, and the catalyst was removed by filtration. The methanol was evaporated yielding pure acid (11.5 g; 94%). The acid (11 g; 0.0286 mol) was suspended in benzene (500 ml), and oxalyl chloride (10ml; 14.5 g; 0.14 mol) and a few drops of DMF were added. The mixture was stirred at room temperature for about 3 hours and evaporated to an oily residue. The oily residue

-53-

was dissolved in chloroform (100ml) and poured into a chloroform (100 ml) solution of O-benzyl-hydroxylamine (14.4 g; 0.117 mol). A white precipitate was formed immediately. After two hours stirring at room temperature the suspension was filtered. Concentrated hydrochloric acid (50 ml) was added to the filtrate and again filtered. The chloroform layer was separated and washed with concentrated hydrochloric acid (3 x 100 ml), water (3 x 100 ml) and dried over anhydrous magnesium sulfate. The chloroform was evaporated yielding pure N-benzyloxyamide (14 g; 82%).

The N-benzyloxyamide (6 g; 0.01 mol) was dissolved in methanol (509 ml) and catalyst (200 mg of 5% Pd-C) was added. The suspension was hydrogenated at room temperature overnight. The catalyst was removed by filtration and the methanol was evaporated yielding pure hydroxamic acid (3.95 g; 95%). The overall yield of 1,3-dimethyl-5,5-di(5-pentyl-n-hydroxylcaboxamide)barbituric acid was 23.4%.

^{1}H NMR (DMSO-d$_6$, 200 MHz), δ 10.30 (s, OH, 2H), 8.60 (broad s, NH, 2H), 3.18 (s, N-CH3, 6H), 1.85 (t, J=7.2 Hz, CH2CONHOH, 4H), 1.80 (t, J=7 Hz, CH2-bar., 4H), 1.39 (m, 4H), 1.10 (m, 8H).

The compound having the structure:

$$2((CH3)2-N-\overset{\overset{O}{\|}}{C})-\overset{\overset{R1}{|}}{C}-(CH2)n-\overset{\overset{R2}{|}}{C}-(\overset{\overset{O}{\|}}{C}-N-(CH3)2))2$$
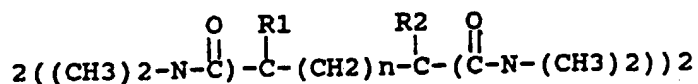
wherein R1 and R2 independently be the same or different and each may is H or a lower alkyl group and n is an integer from 1 to 15 is synthesized as follows. The tetramethylamide (2 g; 6.4 mm 1; for n=2) in DMF (10 ml) was slowly add d to a suspension of sodium hydride (0.9; 38

-54-

mmol) in DMF (50 ml). The mixture was stirred at 80°C for about 30 minutes. Iodomethane (23.3 ml; 53.2 g; 380 mmol) was added to this cool solution at a temperature of about 5°C and the reaction was continued at 60°C overnight. The solvent was evaporated, and the residue was dissolved in water (50 ml). The water solution was extracted with 20% methanol in chloroform (10 x 30 ml). The combined organic extracts were dried over anhydrous magnesium sulfate and evaporated. The product was purified by short column chromatography in acetone. The yields of the alkane-1,1,n,n-tetra-(N,N-dimethylcarboxamide) were for: n=2, 1.3 g (55%); n=3 (52%); n=4, 51%; n=5 (53%); n=6 (53%).

^{1}H NMR (CDCl3, 200 MHz) for compound having n=2, δ 2.94 (s, N-Me, 12 H), 2.87 (s, N-Me, 12H), 1.82 (s, CH2, 4H), 1.32 (s, C-Me, 6H).

-55-

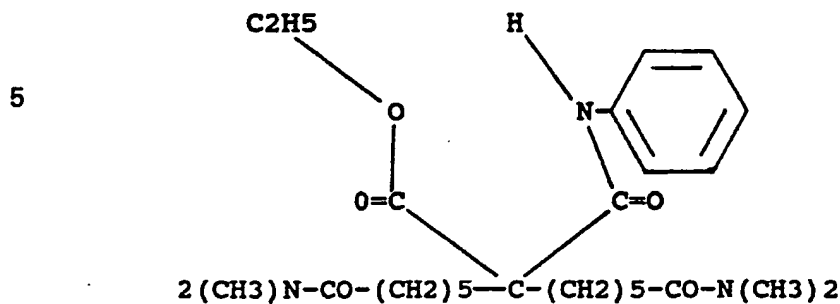The compound having the structure:

5

10
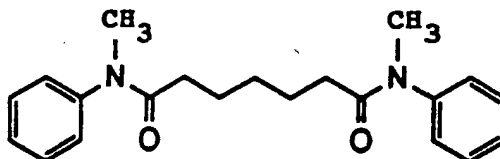
was synthesized as follows. N-methylanyline (7.3 ml; 7.219
g; 67.3 mmol) was slowly added to a solution of pimeloyl
chloride (5 ml; 6.025 g; 30.6 mmol) in chloroform (500 ml).
The suspension was stirred at room temperature for about
15   three hours, and the solid was separated by filtration. The
chloroform   filtrate   was   washed   with   concentrated
hydrochloric acid (3 x 100 ml), water (3 x 100 ml) and dried
over anhydrous magnesium sulfate.   The chloroform was
evaporated, the solid residue was slurried in hexanes and
20   filtered.   The yield   of   pentane-1,5-di(N-methyl-N-
phenylcarboxamide) was 6.3 g (90%).

^{1}H NMR (DMSO-d$_{6}$, 200 MHz), δ 7.30 (m, Ph, 10 H), 3.12 (s, N-
Me, 6H), 1.93 (t, J=7 Hz, CH2CON, 4H), 3.31 (m, 4H), 1.01
25   (m, 2H).

30

35

-56-

The compound having the structure:

C2H5   H
  \   \
   O   N—⟨benzene ring⟩
   | |
  O=C   C=O

        2(CH3)N-CO-(CH2)5—C-(CH2)5-CO-N(CH3)2

was synthesized as follows. To a suspension of sodium hydride (0.24 g; 0.01 mol) in DMF (30ml) at room temperature was added slowly to aniline (1 ml; 1 g; 10.7 mmol) in DMF (5 ml). The mixture was heated at 110 °C for three hours. Diethyl di(N,N-dimethyl-pentamethylenecarboxamide)malonate (4.42 g; 0.01 mol) in DMF (10 ml) was added to the cool reaction mixture. The mixture was stirred at 110°C overnight. The solvent was evaporated. The residue was partitioned between water and chloroform (100-100 ml). The chloroform layer was separated, and the water layer was extracted with 4:1 chloroform-methanol (5 x 50 ml). The combined organic layers were dried over anhydrous magnesium sulfate and evaporated to an oily residue. The product was purified by column chromatography on silica gel in tetrahydrofuran. The yield of undecane-1,11-di(N,N-dimethyl-carboxamide)-6-ethoxycarbonyl-6-(N-phenylcarboxamide) was 2.1 g (43%).

^{1}H NMR (CDCl3, 200 MHz), δ 7.59 (d, J=7.4 Hz, arom. 2H), 7.28 (t, J=7.4 Hz, arom. 2H), 7.26 (s, NH, 1H), 7.10 (t, J=7.4 Hz,arom. 1H), 4.26 (q, J=7 Hz, COOCH2CH3, 2H), 2.99 (s, N-Me, 6H), 2.95 (s, N-Me, 6H), 2.24 (t, J=5.2 Hz, CH2CON, 4H) 1.85 (m, 4H), 1.60 (m, 4H), 1.65 (m, 8H).

-57-

The compounds having the structure (Compounds 13-22, Table IV):

$$\text{HOHN} \overset{\overset{\displaystyle O}{\|}}{\diagup}\text{(CH}_2)_n \overset{\overset{\displaystyle O}{\|}}{\diagdown}\text{NHOH}$$
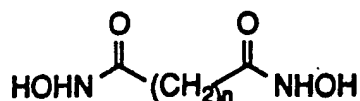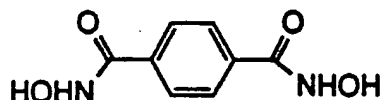
were made as follows. A solution of suberoyl chloride (n=6; 10 ml; 11.72 g; 55.5 mmol) in tetrahydrofuran (50 ml) was slowly added (about 1 hour) to a stirred solution of potassium hydroxide (12.4 g; 22.2 mmol) and hydroxylamine hydrochloride (7.72 g; 111 mmol) in water (200 ml), maintaining the reaction temperature below 5°C. The reaction suspension was stirred an additional two hours at room temperature. The white precipitate (where n= 6-11) was filtered and the filtrate was washed with cold water (5 x 20 ml), acetone (5 x 20 ml) and ether (3 x 50 ml). The product was more than 95% pure by NMR(DMSO). The product can be purified by crystallization from acetone. When n=2-5, the reaction mixture was evaporated. The white crystals were slurried in hot methanol (about 1 liter) and filtered. The filtrate was dried over anhydrous magnesium sulfate and the methanol was evaporated. The white crystals were purified by crystallization from methanol or acetone. The yields of alkane-1,n-di(N-hydroxylcarboxamide) were for n=2, 57%; n=3, 64%; n=5, 71%; n=6, 63%; n=7, 68%; n=8, 78%; n=9, 75%; n=10; 79%; and n=1, 81%.

-58-

The compound having the structure:

$$\text{HOHN} \overset{O}{\overset{\|}{\text{C}}} \text{—} \underset{}{\bigcirc} \text{—} \overset{O}{\overset{\|}{\text{C}}} \text{NHOH}$$

was synthesized as follows. A solution of tetraphthaloyl chloride (5 g; 24 mmol) in tetrahydrofuran (50 ml) was slowly added (about 30 minutes) to a stirred solution of potassium hydroxide (5.52 g; 98.6 mmol) and hydroxylamine hydrochloride (3.4 g; 48.9 mmol) in water (100 ml) and the reaction was maintained at a temperature below 5°C. The reaction suspension was stirred an additional hour at room temperature. The suspension was evaporated, and the solid was slurried in boiling methanol (about 1 liter). The hot methanol suspension was filtered, the filtrate was dried over anhydrous magnesium sulfate and the methanol was evaporated. The solid residue was slurried in ethyl acetate (30 ml). The solid was separated by filtration and washed with ether (5 x 10 ml) and hexanes (5 x 10 ml). The yield of benzene-1,4-di(N-hydroxylcarboxamide) was 3.2 g; (66%).

The compound having the structure:

$$\text{HO-N(CH3)-}\overset{O}{\overset{\|}{\text{C}}}\text{-(CH2)6-}\overset{O}{\overset{\|}{\text{C}}}\text{-N(CH3)-OH}$$
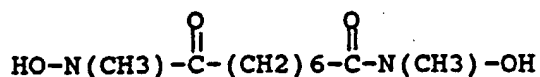
was made by slowly adding a solution of suberoyl chloride (5 g; 23.7 mmol) in tetrahydrofuran (20 ml) to a stirred water solution of potassium hydroxide (5.3 g; 94.8 mmol) and N-methylhydroxylamine hydrochloride (4 g; 47.4 mmol) at about 5°C. The reaction mixture was stirred at room temperature for about one hour. The organic layer was separated and the water layer was extracted with chloroform (5 x 50 ml). The

-59-

combined chloroform layers were dried over anhydrous
magnesium sulfate and evaporated. The product was purified
by crystallization from acetone. The yield of hexane-1,6-
di(N-hydroxyl-N-methylcarboxamide was 2.7 g (46%).

Each compound in Table 1 was assayed 3 or more times to
determine effectiveness as an inducer of MELC (745A-DS19)
cell line. The cell density of MELC in culture for 5 d.
without inducer was 2.0 to 2.6 x 10_6 cells/ml. The cell
density of MELC in culture for 5 d. with inducer was 1.2 to
2.0 x 10_6 cells/ml. Compounds 11 and 12 were dissolved in
absolute ethyl alcohol. The final concentration of ethyl
alcohol in the cultured medium ranged between 0.1 and 3%.
This concentration of ethyl alcohol had no effect on cell
growth of MELC in culture without inducer. All other
compounds were dissolved in culture medium without fetal
calf serum. The indicated optimal concentration represents
the final concentration in the culture medium.

Results

New Hybrid Polar/Apolar Compounds Active as
Cytodifferentiation Agents

In earlier studies (8), the present inventors reported that
fairly high concentrations of certain polar organic solvents
induce MELC to undergo erythroid differentiation. The
question arose as to how the effectiveness of polar
compounds might be increased so that similar concentrations
of these solvent-like molecules could induce
differentiation. Although the mechanisms were not clear,
and are still incompletely understood, the following
hypothesis was considered: perhaps, at the target site of
action, more than one solvent molecule must bind or

-60-

interact. if this were so, the well-known chelate effect could provide more effective compounds. Instead of binding two or more independent solvent molecules, the cellular target might interact more efficiently with a single molecule carrying two or more solvent-like groups. Provided these groups were held in the right relationship one to the other, binding of one would carry the other into the target region providing a high, effective concentration. Such a chelate effect is well precedented; it accounts for the strong binding of metal ions by chelating ligands, and is the basis for much of the catalytic activity of enzyme.

This concept led the present inventors to new effective cytodifferentiating agents. The best studied to data is hexamethylene bisacetamide (HMBA, Table 1, compound 1), consisting of two acetamide molecules linked at nitrogen by a six carbon polyethylene chain (5,9). N-methyl acetamide (Table 1, compound 2), another of the polar organic solvents, was shown to be effective, but only at high concentration (50 mM), whereas HMBA induces erythroid differentiation in MELC at an optimal concentration of 5 mM (8). The present inventors previously showed that the optimum number of methylene groups in the apolar chain is six (27). In the present invention, it was found that a four or five carbon chain is comparably effective if extra carbons are provided as branching methyl groups (Table 1, compounds 3, 4 and 5). This suggests that the important factor is not simply the length of the chain but also the number of hydrophobic hydrocarbon units in it.

Acetamide can also be dimerized by linking the methyl groups of the molecule. Suberic acid bis-N-methylamide (SBDA; Table 1, compound 7), can be thought of as N-methylacetamide linked at the acetyl group by four methylene units. SBDA is comparable in activity as an inducer to HMBA (Figure 1A and

-61-

2). Since the structures of SBDA and HMBA are different, it is likely that the metabolic fates of these two compounds are completely different, and their similarity in effectiveness means that the compounds themselves are the

5   principal active agents, rather than their catabolic products.

HMBA and SBDA have their polar groups separated by identical methylene bridges, and they have a similar ratio of polar to

10   apolar hydrophobic moieties. Many structurally related compounds have been examined, but only a few showed comparable or greater activity (Table 1, compounds 6 through 12). It is clear that the structures of active compounds may differ sufficiently from HMBA to make it likely that

15   they will also display different pharmacokinetics. One of the more active of these hybrid compounds is a dimmer of HMBA. Bishexamethylene triacetamide (BHTA; compound 10) is about 2 fold more active as inducer, on a molar basis, than HMBA (Figure 1C). The most active of the hybrid compounds

20   assayed in this study is one in which two pentamethylene carboxyamides are linked by the dimethyl ester of malonic acid (compound 12). This compound, with 4 exposed polar groups balanced by two apolar pentamethylene domains, is roughly 10 fold more active than HMBA. For example, 0.6 mM

25   compound 12 is about as effective as 5.0 mM HMBA, inducing over 90% of cells to differentiate after 5 d. in culture (Fig. 2).

Polymethylenediamine derivatives carrying propionyl groups

30   instead of the acetyl groups of HMBA are also active, but methoxycarbonyl groups are less effective and bulky pivaloyl groups lead to loss of activity. The present inventors previously showed that HMBA can have a double bond (cis or trans) or a triple bond in the center and retain its

35   activity (27). Replacement of the polymethylene chain with

-62-

a cyclohexane ring leads to inactivity (27), although
compound 9, with a longer chain interrupted by an amide
group, is active.

Diamides of dicarboxylic acids, such as suberic acid, are
active with either one or two methyl groups on each nitrogen
(compounds 7, 8, and 9), but not with a methyl and a
methoxyl substituent, and they are also active with one (but
not two) ethyl groups on each nitrogen. Suberic diamides of
pyrollidine, of morpholine, or of piperazine are inactive.
This shows that there is a limit to the amount of carbon
tolerated on the ends of the polar groups.

These findings indicate that for optimal activity, two, or
even better, three or four uncharged polar groups of limited
bulk must be connected by chains of about 5 to 6 carbons.

Increased Sensitivity of Vincristine-Resistant MELC to Polar/Apolar Compounds

As previously reported, MELC resistant to relatively low
concentrations of vincristine show a marked increase in
sensitivity to HMBA (Fig. 1A) (26). The dose-response of
MELC to several of the new polar compounds identified as
being as or more active than HMBA was examined. The
compounds assayed for inducing activity with vincristine-
resistant MELC include compounds 1, 3, 4, 8, 10, 11 and 12
(Table 1). In each instance, vincristine-resistant MELC
were induced at lower concentrations than were required for
induction of vincristine-resistant MELC were induced more
rapidly than the vincristine-sensitive cells. For the most
active of the compounds, 0.1 mM compound 12 was the optimal
concentration for inducing vincristine-resistant MELC
(VCR.C(2)15). For example, 0.1 mM compound 12 induced only
6% commitment of over 95% of V.C.R.(2) 15 c lls after 2 d.

-63-

and the accumulation of over 80% benzidine-sensitive (DS-19)
MELC, 0.1 mM compound 12 induced only 6% commitment by d. 2
and 4% benzidine reactive cells by d. 5. At concentrations
of compound 12 as low as 0.05, 35% of VCR.C(2)15 became
5   benzidine reactive by d. 2, compared to only 2% of DS-19.

Effect of Polar/Apolar Compounds on MELC Resistant to
Inducer Mediated Terminal Cell Division
10

Several of the new polar/apolar compounds were evaluated as
inducers of MELC cell line DR10, which is resistant to
induction by dimethylsulfoxide and can be induced by HMBA to
accumulate hemoglobin but not commit to terminal cell
15   division (37). Compounds 7, 8 and 10, assayed as inducers
of DR10, caused accumulation of hemaglobin, but not
commitment to terminal cell division. Thus, these new
hybrid polar/apolar compounds are similar to HMBA in their
effect on DR10 cells.
20

Cell cultures were grown in the presence of different
concentrations of Hexamethylenebisacetamide (HMBA)

$$HN-(CH_2)_6-NH$$
$$\mid \qquad\qquad \mid$$
25                                $$C=O \qquad\quad C=O$$
$$\mid \qquad\qquad \mid$$
$$CH_3 \qquad\quad CH_3$$
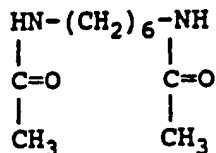
and compound 10, bis-Hexamethylenetriacetamide (IC-135).

30   At 1, 2 and 5 days, the cell densities and the percentage of
cells that were benzidine reactive (B+) were measured.
Table 2 shows the cell densities, B+%, and percent of cells
c mmitt d for cell lines DS19 and V3.17 grown in 1 mM to 5
mM of HMBA and IC-135. Figures 3, 4, 5A and B, and 6A and
35   B are graphical repr sentations of the data pr s nt d in

-64-

Table 2.

Table 3 shows cell counts for days 1, 2 and 5 and percentage
of cells committed and benzidine reactive (B+) at day 5 for
5   cell liens DS-19, V3.17 and DR-10 grown in 5 mM of HMBA and
0.5 to 3 mM of IC-135.

As can be seen in Table 2 and 3 and Figures 3-6, IC-135 is
more   reactive   in   the   tested   cell   lines   at   lower
10   concentrations than HMBA.

15

20

25

30

35

-65-

Discussion

The development of agents which can induce transformed cells
to differentiate and suppress their oncogenicity has
important implications for the treatment of cancer. While
a number of agents have been identified that can induce
tumor cells in vitro to express features of their
differentiated phenotype and to decrease their proliferative
capacity (4,10-24), these agents have generally proved to be
relatively ineffective or two toxic when evaluated in
clinical trials (40).

Among cytodifferentiation agents, the hybrid polar/apolar
compound, HMBA, has been one of the best characterized with
respect to its in vitro inducing activity in MELC and in a
number of other transformed cell lines, as well as for
certain human tumor explants (30). It can trigger a
differentiation program in transformed cells which is
similar to that of their normal lineage (5).

The recent finding that vincristine-resistant MELC are more
sensitive to HMBA and to other active hybrid polar/apolar
compounds provides an approach toward identifying the
mechanisms of HMBA action (26). The lack of a latent period
for inducer-mediated differentiation of vincristine-
resistant cells suggests that an early effect of inducers
may involve alternations (e.g. new proteins, or modification
of existing proteins such as phosphorylation) which are
constitutively expressed in the vincristine-resistant cell
lines, and may, therefore, be identified and characterized.

The observed positive therapeutic responses to HMBA, albeit
largely transient, occurred d spite relatively low serum
concentrations of HMBA (<1 mM), compared to the optimum
d monstrated in vitro (4 t 5 mM) (35,36). The present

-66-

invention provides a new group of hybrid polar/apolar compounds which are as active or even more active, on a molar basis, than HMBA.

5

10

15

20

25

30

35

Table 4

	CPD	Structure	Mol. Weight	Optimal Concentration (μM)	Benzidine Reactive Cells (%)
		$$\begin{array}{ccccc} & H & O & & O & H \\ & | & || & & || & | \\ HO-N-C-(CH2)_n-C-N-OH \end{array}$$			
	13.	n=2	148	ND	
	14.	n=3	162	ND	
	15.	n=4	176	300	60
	16.	n=5	190	40	89
	17.	n=6	202	30	90
	18.	n=7	218	20	75
	19.	n=8	232	20	38
	20.	n=9	246	20	20
	21.	n=10	260	20	26
	22.	n=11	274	20	9
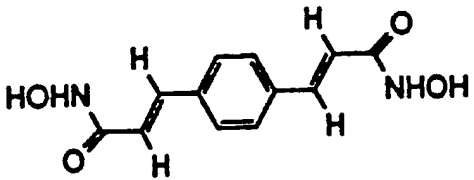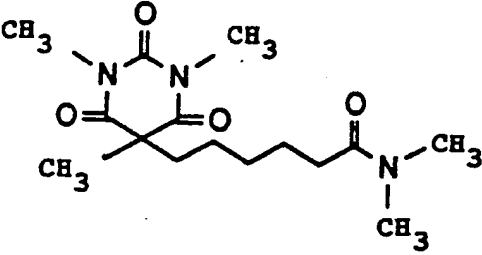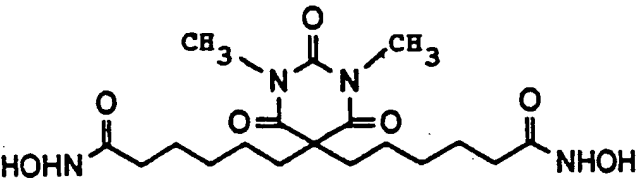
Table 4 (continued)

CPD	Structure	Mol. Weight	Optimal Concentration (μM)	Benzidine Reactive Cells (%)
23.		196	80	88
24.			5	64

Table 4 (continued)
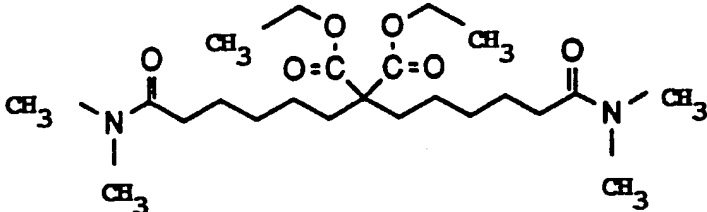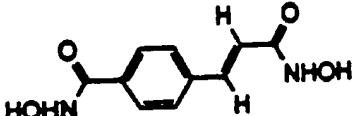
CPD	Structure	Mol. Weight	Optimal Concentration (μM)	Benzidine Reactive Cells (%)
5				
25.				
10				
15		438	600	95
20				
26.				
25				
		424	500	19
30				
35				

-70-

CPD	Structure	Mol. Weight	Optimal Concentration (μM)	Benzidine Reactive Cells (%)
27.				

CH_3

CH_3

O

N   N

O   O

CH_3

O

N—CH_3

CH_3

| | | 311 | 5000 | 85 |

28.

CH_3 CH_3

O

N   N

O   O

HOHN

O

O

NHOH

| | | 414 | 600 | 85 |

CPD	Structure	Mol. Weight	Optimal Concentration (μM)	Benzidine Reactive Cells (%)

5

29.

| | | 442.60 | 600 | 95 |

15

30.

20

25

| | | | 2.5 | 40 |

30

35

-72-

References:

1. Sporn, M.B., Roberts, A.B., Driscoll, J.S. (1985) in
    Cancer: Principles and Practice of Oncology, eds.
    Hellman, S., Rosenberg, S.A. & DeVita, V.T., Jr., Ed.
    2, (J.B. Lippincott, Philadelphia), P. 49.

2. Breitman, T.R., Selonick, S.E., and Collins, S.J.
    Induction of differentiation of the human promyelocytic
    leukemia cell line (HL-60) by retinoic acid. Proc.
    Natl. Acad. Sci. USA., 77:2936-2940, 1980.

3. Olsson, I.L., and Breitman, T.R. Induction of
    differentiation of the human histiocytic lymphoma cell
    line U.937 by retinoic acid and cyclic adenosine 3':5'-
    monophosphate-inducing agents. Cancer Res., 42:3924-
    3927, 1982.

4. Schwartx, E.L. & Sartorelli, A.C. (1982) Cancer Res.,
    42, 2651-2655.

5. Marks, P.A., Sheffery, M. & Rifkind, R.A. (1987) Cancer
    Res. 47, 659.

6. Sachs, L. (1978) Nature (Lond.) 274, 535.

7. Friend, C., Scher, W., Holland, J.W. & Sato, T. (1971)
    Proc. Natl. Acad. Sci. (USA), 68, 378-382.

8. Tanaka, M., Levy, J., Terada, M., Breslow, R., Rifkind,
    R.A. & Marks, P.A. (1975) Proc. Natl. Acad. Sci. (USA),
    72, 1003-1006.

9. Reuben, R.C., Wife, R.L., Breslow, R., Rifkind, R.A. &
    Marks, P.A. (1976) Proc. Natl. Acad. Sci. (USA), 73,

-73-

862-866.

10. Abe, E., Miyaura, C., Sakagami, H., Takeda, M., Konno, K., Yamazaki, T., Yoshika, S. & Suda, T. (1981) Proc. Natl. Acad. Sci. (USA), 78, 4990-4994.

11. Schwartz, E.L., Snoddy, J.R., Kreutter, D., Rasmussen, H. & Sartorelli, A.C. (1983) Proc. Am. Assoc. Cancer Res., 24, 18.

12. Tanenaga, K., Hozumi, M. & Sakagami, Y. (1980) Cancer Res., 40, 914-919.

13. Lotem, J. & Sachs, L. (1975) Int. J. Cancer, 15, 731-740.

14. Metcalf, D. (1985) Scienct, 229, 16-22.

15. Scher, W., Scher, B.M. & Waxman, S. (1983) Exp. Hematol., 11, 490-498.

16. Scher, W., Scher, B.M. & Waxman, S. (1982) Biochem. & Biophys. Res. Comm., 109, 348-354.

17. Huberman, E. & Callaham, M.F. (1979) Proc. Natl. Acad. Sci. (USA), 76, 1293-1297.

18. Lottem, J. & Sachs, L. (1979) Proc. Natl. Acad. Sci. (USA), 76, 5158-5162.

19. Terada, M., Epner, E., Nudel, U., Salmon, J., Fibach, E., Rifkind, R.A. & Marks, P.A. (1978) Proc. Natl. Acad. Sci. (USA), 75, 2795-2799.

20. Morin, M.J. & Sart relli, A.C. (1984) Cancer Res., 44,

-74-

2807-2812.

21. Schwartz, E.L., Brown, B.J., Nierenberg, M., Marsh,
     J.C. & Sartorelli, A.C. (1983) Cancer Res., 43, 2725-
     2730.

22. Sugano, H., Furusawa, M., Kawaguchi, T. & Ikawa, Y.
     (1973) Bibl. Hematol., 39, 943-954.

23. Ebert, P.S., Wars, I. & Buell, D.N. (1976) Cancer Res.,
     36, 1809-1813.

24. Hayashi, M., Okabe, J. & Hozumi, M. (1979) Gann, 70,
     235-238.

25. Fibach, E., Reuben, R.C., Rifkind, R.A. & Marks, P.A.
     (1977) Cancer Res., 37, 440-444.

26. Melloni, E., Pontremoli, S., Damiani, G., Viotti, P.,
     Weich, N., Rifkind, R.A. & Marks, P.A. (1988) Proc.
     Natl. Acad. Sci. (USA), 85, 3835-3839.

27. Reuben, R., Khanna, P.L., Gazitt, Y., Breslow, R.,
     Rifkind, R.A. & Marks, P.A. (1978) J. Biol. Chem., 253,
     4214-4218.

28. Marks, P.A. and Rifkind, R.A. Hexamethylene Biscetamide
     Induced   Differentiation   of   Transformed   Cells:
     Molecular   and   Cellular   Effects   and   Therapeutic
     Application.   International Journal of Cell Cloning,
     6:230-240, 1988.

29. Melloni, E., Pontremoli, S., Michetti, M., Sacco, O.,
     Cakiroglu, A.G., Jackson, J.F., Rifkind, R.A. & Marks,
     P.A. Proc. Natl. Acad. Sciences (USA) 84, 5282-5286,

-75-

1987.

30. Marks, P.A. & Rifkind, R.A. (1984) Cancer, 54, 2766-
     2769.

31. Egorin, M.J., Sigman, L.M. VanEcho, D.A., Forrest, A.,
     Whitacre, M.Y. & Aisner, J. (1987) Cancer Res., 47,
     617-623.

32. Rowinsky, E.W., Ettinger, D.S., Grochow, L.B.,
     Brundrett, R.B., Cates, A.E. & Donehower, R.C. (1986)
     J. Clin. Oncol., 4, 1835-1844.

33. Rowinsky, E.L. Ettinger, D.S., McGuire, W.P., Noe,
     D.A., Grochow, L.B. & Donehower, R.C. (1987) Cancer
     Res., 47, 5788-5795.

34. Callery, P.S., Egorin, M.J., Geelhaar, L.A. & Nayer,
     M.S.B. (1986) Cancer Res., 46, 4900-4903.

35. Young, C.W. Fanucchi, M.P., Walsh, T.B., Blatzer, L.,
     Yaldaie, S., Stevens, Y.W., Gordon, C., Tong, W.,
     Rifkind, R.A. & Marks, P.A. (1988) Cancer Res., 48,
     7304-7309.

36. Andreeff, M., Young, C., Clarkson, B., Fetten, J.,
     Rifkind, R.A. & Marks, P.A. (1988) Blood, 72, 186a.

37. Ohta, Y., Tanaka, M., Terada, M., Miller, O.J., Bank,
     A., Marks, P.A. & Rifkind, R.A. (1976) Proc. Natl.
     Acad. Sci. (USA) 73, 1232-1236.

38. Cartledge, F.K. & Nguyen, T.B. (1986) J. Org. Chem.,
     51, 2206-2210.

39. Hoffmann-LaRoche and Co., A.G. British Patent 1. 138-
     529 (1969).

40. Dmitrovsky, E., Markman, M. & Marks, P.A. (1989) in
     <u>Cancer Chemotherapy and Biological Therapy</u>, Annual 11,
     eds., D.L. Longo, H.M. Pinedo, B.A. Chabner, eds.,
     Excepta Medica, Publisher, in press.

-77-

1.   A compound having the structure:

$$[R\text{-}A]\text{-}B\text{-}A_1\text{-}B_1\text{-}[A_2\text{-}B_2\text{-}]_a[A_3\text{-}B_3\text{-}]_b[A_4\text{-}R_1]$$

wherein each A, A_1, A_2, A_3, and A_4 represent a polar group which comprises a nitrogen, sulfur or oxygen atom and wherein each of A, A_1, A_2, A_3, and A_4 may independently be the same as, or different from, the others of A, A_1, A_2, A_3, and A_4;

wherein each of R and R_1 is a hydrogen atom; a lower alkyl, alkenyl, or alkynyl group; or a group having the structure:

$$\begin{array}{c} R_2 \\ | \\ \text{-N} \\ | \\ R_3 \end{array}$$

wherein each of R_2 and R_3 being a hydrogen atom or a lower alkyl, alkenyl, or alkynyl group; and wherein each of R, R_1, R_2 and R_3 may independently be the same as, or different from, the others of R, R_1, R_2 and R_3;

wherein each of [R-A] and $[A_4\text{-}R_1]$ have a dipolar moment greater than about 2.7 Debye units;

wherein each of B, B_1, B_2, and B_3 represents a nonpolar group which comprises at least 4 atoms in a chain, the termini of which chains are attached to A and A_1, A_1 and A_2, A_2 and A_3, and A_3 and A_4, respectively; wherein each such atom is oxygen, nitrogen, carbon, or sulfur and wherein each of B, B_1, B_2, and B_3 may independently

-78-

be the same or different from, each of B, B_1, B_2, and B_3;

and wherein each of a and b is independently 0 or 1.

5

2.   A compound of claim 1 having the structure:

$$CH_3CH_2O$$

$$(CH3)_2-N-C-(CH2)_5-C-(CH2)_5-C-N-(CH3)_2$$

$$O = C \quad OCH_2CH_3$$

10

3.   A compound of claim 1, wherein each of A, A_1, A_2, A_3 and A_4 is a carbonyl radical or a bivalent radical of an amide, a sulfoxide, or an amine oxide.

15

4.   A compound of claim 1, wherein each of A, A_1, A_2, A_3 and A_4 is a carbonyl radical or a bivalent radical of an amide.

20

5.   A compound of claim 4, wherein all of A, A_1, A_2, A_3, and A_4 are the same.

25

30

35

-79-

6.   A compound of claim 5, wherein all of A, A_1, A_2, A_3 and A_4 have the structure:

$$
\begin{array}{c}
\text{-N-} \\
| \\
\text{C=O} \\
| \\
R_4
\end{array}
$$
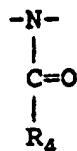
wherein R_4 is a hydrogen atom or a lower alkyl or alkenyl group.

7.   A compound of claim 4, wherein all of A, A_1, A_2, A_3 and A_4 are a carbonyl radicals.

8.   A compound of claim 1, wherein R and R_1 are the same and are hydrogen atoms, methyl groups, ethyl groups or groups having the structure:

$$
\begin{array}{c}
R_2 \\
| \\
\text{-N} \\
| \\
R_3
\end{array}
$$
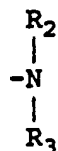
each of R_2 and R_3 each is hydrogen atom, a methyl group an ethyl group and being the same or different.

9.   A compound of claim 8, wherein all of A, A_1, A_2, A_3, and A_4 are carbonyl radicals and R and R_1 are:

$$
\begin{array}{c}
R_2 \\
| \\
\text{-N} \\
| \\
R_3
\end{array}
$$
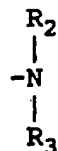
10.   A compound of claim 1, wherein each of B, B_1, B_2, and B_3 further comprises a nonpolar substituent attached to

-80-

an atom of a chain.

11. A compound of claim 1, wherein each of B, B_1, B_2, and
     B_3 comprises 4 to 7 atoms connected in a chain.

12. A compound of claim 1, wherein each of B, B_1, B_2, and
     B_3 may comprise a hydrocarbon chain.

13. A compound of claim 1 having the structure:
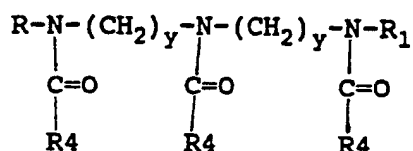
$$R-N-(CH_2)_y-N-(CH_2)_y-N-R_1$$
$$\begin{array}{ccc} C=O & C=O & C=O \\ R4 & R4 & R4 \end{array}$$

     wherein each of R, R_1, and R_4 is a hydrogen atom or a
     methyl, ethyl, or propyl group, R and R1 are the same
     or different; and wherein y is 4, 5, or 7.

14. A compound of claim 13, wherein R and R_1 are hydrogen
     atoms, each R4 is an ethyl group, and y is 4, 5 or 6.

15. A compound of claim 13 having the structure:

$$H-N-(CH_2)_y-N-(CH_2)_y-N-H$$
$$\begin{array}{ccc} C=O & C=O & C=O \\ CH_3 & CH3 & CH_3 \end{array}$$
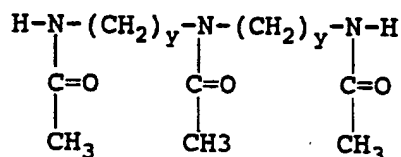
     wherein y is 4, 5 or 6.

16. A compound of claim 15 having the structure:
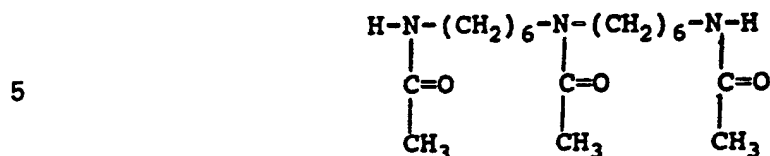
$$H-N-(CH_2)_6-N-(CH_2)_6-N-H$$
$$|\quad\quad\quad\quad|\quad\quad\quad\quad|$$
$$C=O\quad\quad C=O\quad\quad C=O$$
$$|\quad\quad\quad\quad|\quad\quad\quad\quad|$$
$$CH_3\quad\quad CH_3\quad\quad CH_3$$

17. A compound of claim 1, having the structure:

$$R_2-N-\overset{\overset{O}{\|}}{C}-(CH_2)_y-A_1-(CH_2)_{y1}-\overset{\overset{O}{\|}}{C}-N-R_3$$
$$|\qquad\qquad\qquad\qquad\qquad\qquad\qquad|$$
$$R_3\qquad\qquad\qquad\qquad\qquad\qquad R_3$$
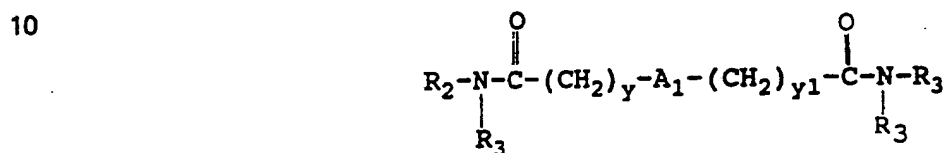
wherein each of R2 and R3 is a hydrogen atom or a methyl, ethyl of propyl group and is the same or different, and wherein each of y and y1 is independently 4, 5, 6 or 7.

18. A compound of claim 17, wherein R_2 is a hydrogen atom, R_3 is an ethyl· group and each of y and y1 is independently 4, 5 or 6.

19. A compound of claim 17, wherein A1 is

$$-\overset{\overset{O}{\|}}{C}-\ ,\qquad -CH-,\qquad\qquad or\qquad -\overset{\overset{O}{\|}}{C}-NR5—.$$
$$\qquad\qquad |$$
$$\qquad\qquad C=O$$
$$\qquad\qquad |$$
$$\qquad\qquad R5-N-R5$$
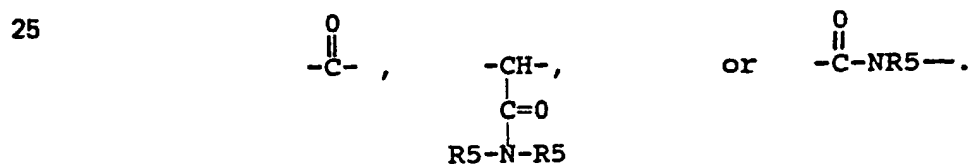
wherein R5 is a hydrogen atom or a methyl, ethyl or propyl group.

-82-

20. A compound of claim 19 having the structure:

$$(CH_3)_2-N-\overset{\overset{\displaystyle O}{\|}}{C}-(CH_2)_5-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle N-(CH_3)_2}{|}}{\underset{\displaystyle C=O}{|}}}-(CH_2)5-\overset{\overset{\displaystyle O}{\|}}{C}-N-(CH_3)_2$$
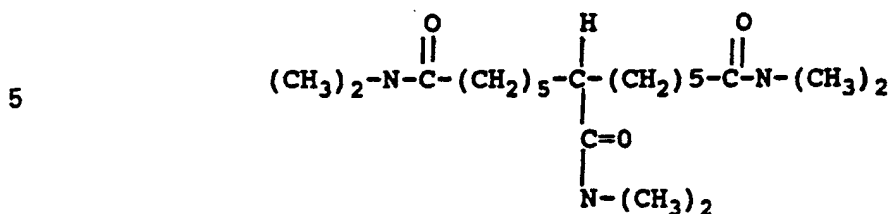
21. A compound of claim 18 having the structure:

$$CH_3-\overset{\overset{\displaystyle}{\underset{\displaystyle CH_3}{|}}}{N}-\overset{\overset{\displaystyle O}{\|}}{C}-(CH2)y-A_1-(CH_2)y_1-\overset{\overset{\displaystyle O}{\|}}{C}-\overset{\overset{\displaystyle}{\underset{\displaystyle CH_3}{|}}}{N}-CH_3$$
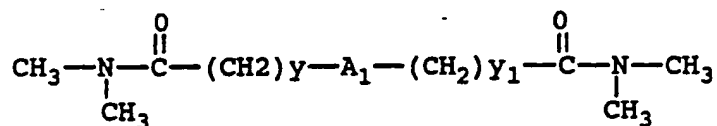
wherein each of y and y_1 is independently 5 or 6.

22. A compound of claim 21, wherein A_1 is:

$$-\overset{\overset{\displaystyle O}{\|}}{C}-, \qquad -\overset{\overset{\displaystyle}{\underset{\underset{\displaystyle NH_2}{|}}{\underset{\displaystyle C=O}{|}}}}{CH}-, \qquad or \quad -\overset{\overset{\displaystyle O}{\|}}{C}-NH-$$
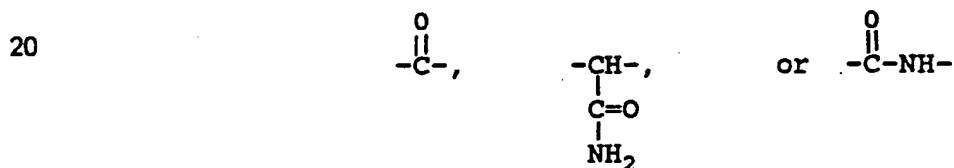
23. A compound having the structure:

$$(CH_3)_2N-\overset{\overset{\displaystyle O}{\|}}{C}-(CH_2)_5 \qquad (CH_2)_5-\overset{\overset{\displaystyle O}{\|}}{C}-N(CH_3)_2$$
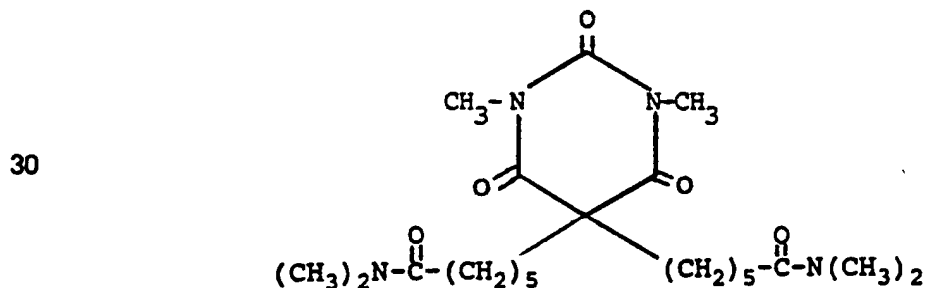
24. A compound having the structure:

5
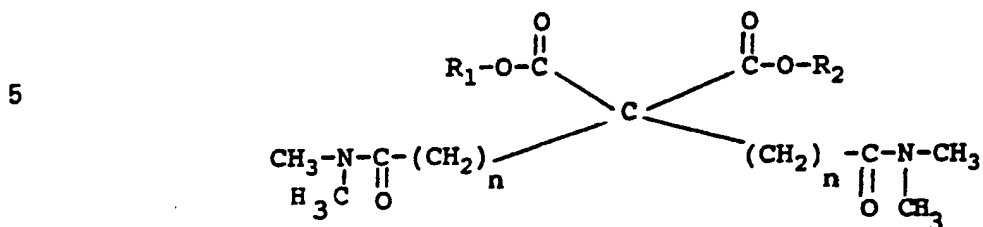
wherein n is an integer greater than 1; and wherein R1
and R2 may be the same or different and each is a lower
alkyl group.

10

25. A compound having the structure:

15

20
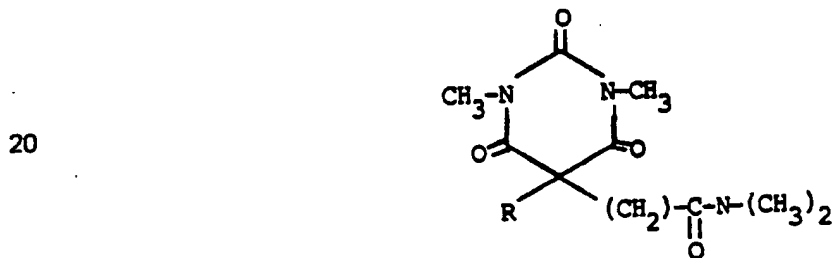
25

wherein R is H or a lower alkyl group.

30

35

-84-

26. A compound having the structure:
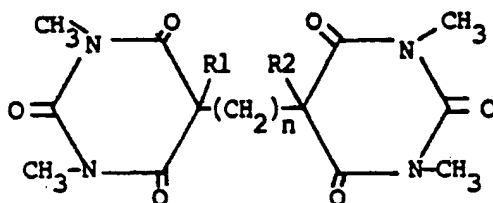
wherein n is an integer which is greater than 1 and R_1 and R_2 may be the same or different and each is H or a lower alkyl group.

27. A compound having the structure:
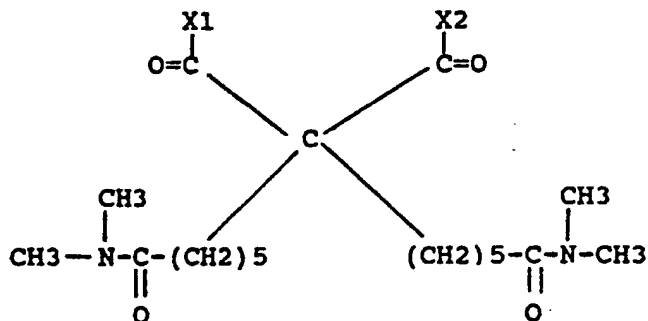
wherein n is an integer which is greater than 1 and wherein X1 and X2 may independently be the same or different and each is $N(CH_3)_2$, NH-phenyl, $O-C_2H_5$ or $HNCH_3$.

28. A compound having the structure:
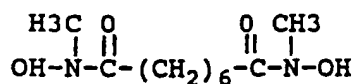
29. A compound having the structure:
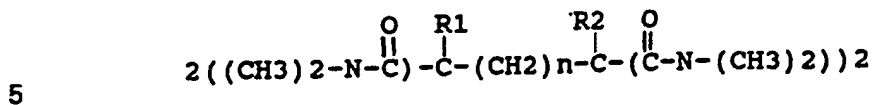
$$2((CH_3)_2-N-\overset{\overset{\textstyle O}{\|}}{C})-\overset{\overset{\textstyle R1}{|}}{C}-(CH_2)n-\overset{\overset{\textstyle R2}{|}}{C}-(\overset{\overset{\textstyle O}{\|}}{C}-N-(CH_3)_2))_2$$

wherein R1 and R2 may be the same or different and each is H or a lower alkyl group;

and n is an integer from 1 to 15.

30. A compound having the structure:

$$\begin{array}{cc} \overset{\textstyle R3}{\underset{\textstyle R2-N}{|}}\ O & \overset{\textstyle R4}{\underset{\textstyle N-R5}{|}} \\ R1-N-\overset{\overset{\textstyle \|}{C}}{}-(X)n-\overset{\overset{\textstyle O}{\|}}{C}-N-R6 \end{array}$$

wherein R1, R2, R3, R4, R5, R6 may independently be the same or different from each other and is H or a lower alkyl group;

wherein X is methyl or phenyl; and

n is an integer from 1 to about 15.

31. A compound having the structure:

$$\begin{array}{c} R_2-N \text{———} N-R_1 \\ | \qquad\qquad | \\ O=C \qquad\quad C=O \\ \diagdown \qquad\quad \diagup \\ C \\ \diagup \quad \diagdown \\ \underset{\overset{\displaystyle \|}{O}}{CH_3-N-C-(CH_2)_5} \quad \underset{\overset{\displaystyle \|}{O}}{(CH_2)_5-C-N-CH_3} \\ \overset{|}{CH_3} \qquad\qquad \overset{|}{CH_3} \end{array}$$

wherein R1 and R2 may be the same or different and is H or CH3.

32. A compound having the structure:

-86-

$$2HC \underline{\hspace{3cm}} CH_2$$

R2-N   N-R1

C   C

O   O

C

CH₃ - N — C-(CH₂)₅   (CH2)5-C-N-CH3

CH3   O   O   CH3

wherein R₁ and R₂ may be the same or different and is
H or CH₃.

33. A compound having the structure:

$$HO-N - C -X - C - N - OH$$

with R₁ O / O R₂

wherein X has the structure:

-CH=CH-⟨ ⟩-CH=CH-,

-C≡C-⟨ ⟩-C≡C-,   ⟨ ⟩,

⟨ ⟩-C≡C-,

⟨ ⟩-CH=CH-

-CH₂\   H
     \C = C/
H/   \CH₂-

-(CH2) ⟨ ⟩-(CH2) - ,   or ⟨ ⟩-⟨ ⟩-

-87-

=CH=CH=CH=CH=;

wherein R_1 and R_2 are the same or different and is H or lower alkyl group.

34.   A compound having the structure:

$$O=C \begin{array}{c} \overset{CH_3}{\underset{|}{N}} \overset{O}{\overset{\|}{C}} \\ \\ \underset{|}{\overset{|}{N}} \overset{C}{\underset{\|}{C}} \\ CH3 \quad O \end{array} \overset{\displaystyle C}{\underset{\displaystyle (CH_2)n-\overset{\|}{\underset{\|}{C}}-\overset{R2}{\underset{|}{N}}-R4}{\overset{(CH_2)n-\overset{O}{\overset{\|}{C}}-\overset{R1}{\underset{|}{N}}-R3}{}}}$$

wherein R1 and R1 may independently be the same or different and each may be H or a lower alkyl group;

wherein R3 and R4 may independently be the same or different and each may be CH3 or OH; and

wherein n is 5 or 6.

-88-

35. A compound having the structure:

$$\text{(CH3)2-N-}\overset{\displaystyle O}{\overset{\|}{\text{C}}}\text{-(CH2)6-}\overset{\displaystyle \overset{R1}{|}}{\underset{\underset{R2}{|}}{\overset{\text{C=O}}{\underset{\text{C=O}}{\text{C}}}}}\text{-(CH2)6-}\overset{\displaystyle O}{\overset{\|}{\text{C}}}\text{-N-(CH3)2}$$

wherein R_1 and R_2 is the same or different and is hydrogen, lower alkyl, alkenyl, alkynyl, an amide or hydroxyamide.

36. A compound having the structure:

⟨◯⟩-N(CH3)-CO-(CH2)5-CO-N(CH3)-⟨◯⟩

37. A compound having the structure:

$$\underset{\text{HO-NH-CO-(CH2)5}}{\overset{\text{H5C2-O}}{\overset{|}{\underset{\text{O=C}}{}}}}\overset{\text{C}}{\underset{\text{(CH2)5-CO-NH-OH}}{}}\overset{\text{O-C2H5}}{\overset{|}{\underset{\text{C=O}}{}}}$$

38. A compound having the structure:

⟨◯⟩-N(CH3)-CO—(CH2)5—CO-N(CH3)-⟨◯⟩ .

39. A compound having the structure:

-89-

2(CH3)N-CO-(CH2)5—C—(CH2)5-CO-N(CH3)2

40.   A   method   of   selectively   inducing   terminal
differentiation   of   neoplastic   cells   and   thereby
inhibiting proliferation of such cells which comprises
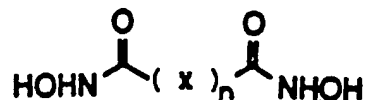contacting the cells under suitable conditions with an
amount of a compound effective to selectively induce
terminal   differentiation,   the   compound   having   the
structure:

$$[R-A]-B-A_1-B_1-[A_2-B_2-]_a[A_3-B_3-]_b[A_4-R_1]$$

wherein each A, A_1, A_2, A_3, and A_4 represent a polar
group which comprises a nitrogen, sulfur or oxygen atom
and   wherein   each   of A, A_1, A_2, A_3,   and A_4 may
independently be the same as, or different from, the
others of A, A_1, A_2, A_3, and A_4;

wherein each of R and R_1 is a hydrogen atom; a lower
alkyl, alkenyl, or alkynyl group; or a group having the
structure:

$$\begin{array}{c} R_2 \\ | \\ -N \\ | \\ R_3 \end{array}$$

wherein each of R_2 and R_3 being a hydrogen atom or a
lower   alkyl,   alkenyl,   or   alkynyl   group;   and   wherein
each of R, R_1, R_2 and R_3 may independently be the same
as, or diff rent from, the others of R, R_1, R_2 and R_3;

-90-

wherein each of [R-A] and [A$_4$-R$_1$] have a dipolar moment greater than about 2.7 Debye units;

5

wherein each of B, B$_1$, B$_2$, and B$_3$ represents a nonpolar group which comprises at least 4 atoms in a chain, the termini of which chains are attached to A and A$_1$, A$_1$ and A$_2$, A$_2$ and A$_3$, and A$_3$ and A$_4$, respectively; wherein each such atom is oxygen, nitrogen, carbon, or sulfur and wherein each of B, B$_1$, B$_2$, and B$_3$ may independently

10

be the same or different from each of B, B$_1$, B$_2$, and B$_3$;

and wherein each of a and b is independently 0 or 1.

15

20

25

30

35

41. A method of claim 40, wherein the compound has the structure:

$$CH_3CH_2O - C(=O) \quad | \quad (CH_3)_2-N-\overset{O}{\overset{\|}{C}}-(CH_2)_5-\overset{}{C}-(CH_2)_5-\overset{O}{\overset{\|}{C}}-N-(CH_3)_2 \quad | \quad \overset{}{C}(=O)-OCH_2CH_3$$
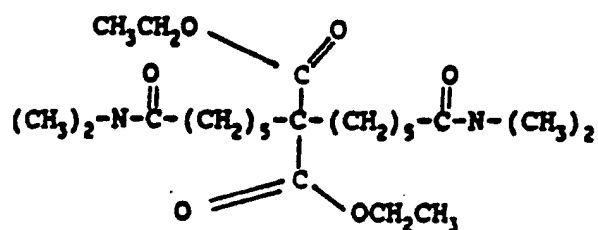
42. A method of claim 40, wherein the contacting is effected continuously for at least 48 hours.

43. A method of claim 40, wherein the compound has the structure:
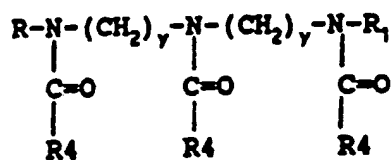
$$R-N-(CH_2)_y-N-(CH_2)_y-N-R_1 \quad | \quad\quad | \quad\quad | \quad C=O \quad\quad C=O \quad\quad C=O \quad | \quad\quad | \quad\quad | \quad R4 \quad\quad R4 \quad\quad R4$$

wherein each of R, R_1, and R_4 is a hydrogen atom or a methyl, ethyl or propyl group, R and R_1 are the same and R and R_4 are the same or different; and wherein y is 4, 5, 6 or 7.

-92-

44. A method of claim 43, wherein the compound has the
    structure:

5

$$\text{H-N-(CH}_2)_6\text{-N-(CH}_2)_6\text{-N-H}$$
$$\begin{array}{ccc} | & | & | \\ C=O & C=O & C=O \\ | & | & | \\ CH_3 & CH_3 & CH_3 \end{array}$$
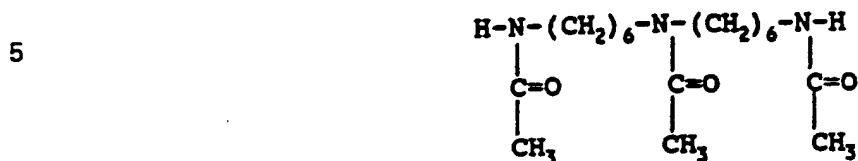
45. A method of claim 43, wherein the amount of the
10 compound is from about 0.5mM to about 25mM.

46. A method of claim 40, wherein the compound has the
    structure:
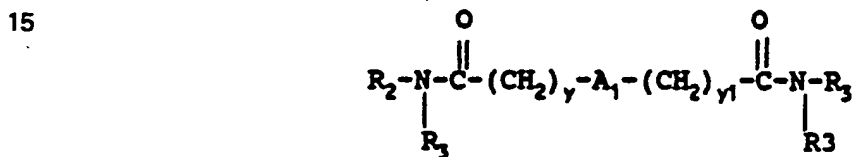
15

$$R_2\text{-N-}\overset{\displaystyle O}{\overset{\|}{C}}\text{-(CH}_2)_y\text{-A}_1\text{-(CH}_2)_{y1}\text{-}\overset{\displaystyle O}{\overset{\|}{C}}\text{-N-R}_3$$
$$\begin{array}{cc} | & | \\ R_3 & R3 \end{array}$$

20 wherein each of R_2 and R_3 is a hydrogen atom or a
    methyl, ethyl or propyl group and each being the same
    or different; wherein A_1 is

25

$$\overset{\displaystyle O}{\overset{\|}{-C-}}, \quad -CH-, \quad \text{or} \quad \overset{\displaystyle O}{\overset{\|}{-C-NH-}};$$
$$\begin{array}{c} | \\ C=O \\ | \\ NH_2 \end{array}$$
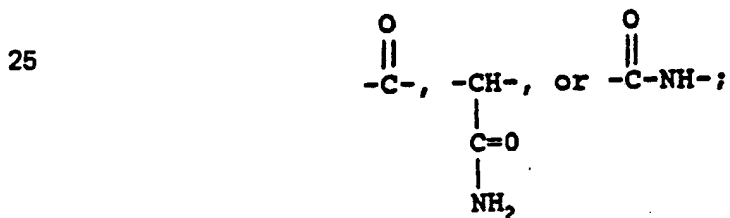
30 and wherein each of y and y_1 is independently 5, 6 or
    7.

35

47. A method of claim 46, wherein the compound has the structure:

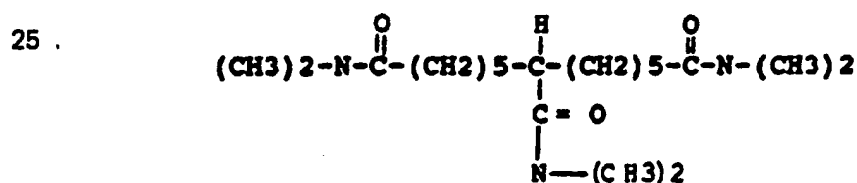$$(CH_3)_2-N-\overset{\overset{\displaystyle O}{\|}}{C}-(CH_2)_5-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle N-(CH_3)_2}{|}}{\underset{\displaystyle C=O}{\underset{|}{C}}}}-(CH_2)5-\overset{\overset{\displaystyle O}{\|}}{C}-N-(CH_3)_2$$
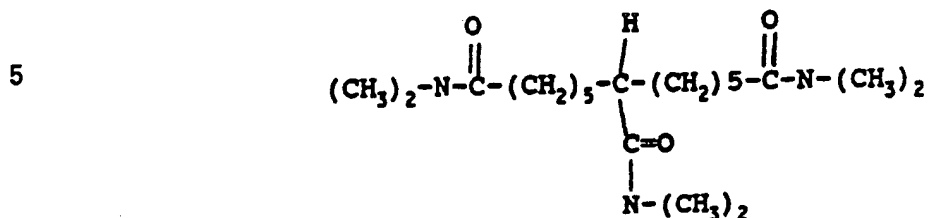
48. A method of claim 47, wherein the amount of the compound is from about 0.1 to about 10 mM.

49. A method of selectively inducing terminal differentiation of neoplastic cells and thereby inhibiting proliferation of such cells which comprises contacting the cells under suitable conditions with an amount of a compound of claims 23-39, effective to selectively induce terminal differentiation.

50. A method of selectively inducing terminal differentiation of neoplastic cells and thereby inhibiting proliferation of such cells which comprises contacting the cells under suitable conditions with an amount of a compound effective to selectively induce terminal differentiation, the compound having the structure:

$$HOHN\overset{\overset{\displaystyle O}{\|}}{\underset{}{C}}(X)_n\overset{\overset{\displaystyle O}{\|}}{\underset{}{C}}NHOH$$

wherein X is phenyl or methyl and n is an integer from 1 to 15.

-94-

51. A method of claim 49 or 50 wherein the effective amount of the compound is from about 2μm to about 6000 μm.

5

52. A method of claim 51, wherein the contacting is effected continuously for at least 48 hours.

53. A method of treating a patient having a tumor characterized by proliferation of neoplastic cells which comprises administering to the patient an amount of a compound effective to selectively induce terminal differentiation of such neoplastic cells and thereby inhibit their proliferation, the compound having the structure:

10

15

$[R-A]-B-A_1-B_1-[A_2-B_2-]_a[A_3-B_3-]_b[A_4-R_1]$

wherein each of A, A_1, A_2, A_3, and A_4 represent a polar group which comprises a nitrogen, sulfur or oxygen atom and wherein each of A, A_1, A_2, A_3, and A_4 may independently be the same as, or different from, the others of A, A_1, A_2, A_3, and A_4;

20

wherein each of R and R_1 is a hydrogen atom; a lower alkyl, alkenyl, or alkenyl group; or a group having the structure:

25

```
        -N-
         |
        C=O
         |
        R₄
```
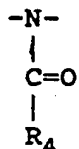
30

wherein each of R_2 and R_3 being a hydrogen atom or a lower alkyl, alkenyl, or alkynyl group; and wherein each of R, R_1, R_2 and R_3;

wherein each of [R-A] and [A_4-R_1] have a dipolar moment

35

-95-

greater than about 2.7 Debye units;

wherein each of B, B_1, B_2, and B_3 represents a nonpolar
group of which comprises least 4 atoms in a chain, the
termini of which chains are attached to A and A_1, A_1
and A_2 A_2 and A_3, and A_3 and A_4, respectively; wherein
each such atom is oxygen, nitrogen, carbon, or sulfur
and wherein each of B, B_1, B_2, and B_3 may independently
be the same as, or different from the others of B, B_1,
B_2 and B_3;

and wherein each of a and b is independently 0 or 1.

54.   A method of claim 53, wherein the compound has the
structure:
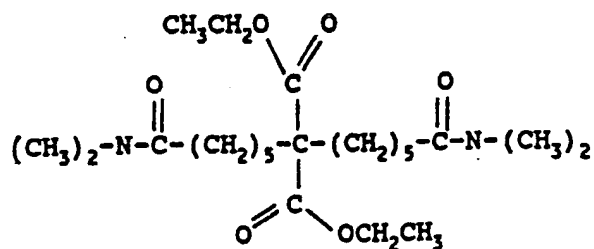
$$(CH_3)_2-N-\overset{\overset{O}{\|}}{C}-(CH_2)_5-\overset{\overset{CH_3CH_2O}{\diagdown}\overset{O}{\diagup}C}{\underset{\overset{C}{O}\diagdown_{OCH_2CH_3}}{C}}-(CH_2)_5-\overset{\overset{O}{\|}}{C}-N-(CH_3)_2$$

55.   A method of claim 53, wherein the compound has the
structure:

$$\begin{array}{c} R-N-(CH_2)_y-N-(CH_2)_y-N-R_1 \\ | \qquad\quad | \qquad\quad | \\ C=O \qquad C=O \qquad C=O \\ | \qquad\quad | \qquad\quad | \\ R4 \qquad\quad R4 \qquad\quad R4 \end{array}$$
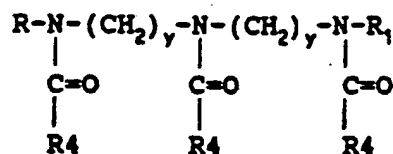
wherein each of R, R_1, and R_4 is a hydrogen atom or a
methyl, ethyl or propyl group, R and R1 are the same
and R and R4 are the same or different; and wherein y
is 4, 5, 6 or 7.

56.   A method of claim 55, wherein the compound has the
structure:

$$\begin{array}{c} H-N-(CH2)_6-N-(CH_2)_6-N-H \\ | \qquad\quad | \qquad\quad | \\ C=O \qquad C=O \qquad C=O \\ | \qquad\quad | \qquad\quad | \\ CH_3 \qquad\quad CH_3 \qquad\quad CH_3 \end{array}$$
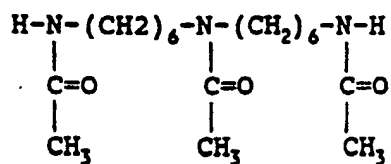
-97-

57. A method of claim 51, wherein the compound has the structure:
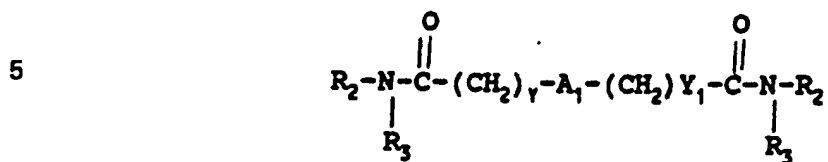
$$R_2-N-\overset{\overset{\displaystyle O}{\|}}{C}-(CH_2)_y-A_1-(CH_2)Y_1-\overset{\overset{\displaystyle O}{\|}}{C}-N-R_2$$
$$\underset{\displaystyle R_3}{|} \qquad\qquad\qquad \underset{\displaystyle R_3}{|}$$

wherein each of R_2 and R_3 is a hydrogen atom or a methyl, ethyl or propyl group and each being the same or different; wherein A_1 is

$$-\overset{\overset{\displaystyle O}{\|}}{C}-, \quad -CH-, \quad or \quad -\overset{\overset{\displaystyle O}{\|}}{C}-NH-;$$
$$\underset{\displaystyle \underset{\displaystyle NH_2}{|}}{\overset{\displaystyle |}{C=O}}$$
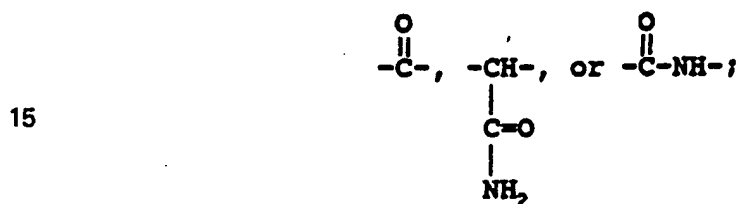
and wherein each of y and y_1 is independently 5, 6 or 7.

58. A method of claim 57, wherein the compound has the structure:

$$(CH3)2-N-\overset{\overset{\displaystyle O}{\|}}{C}-(CH2)5-\overset{\overset{\displaystyle H}{|}}{C}-(CH2)5-\overset{\overset{\displaystyle O}{\|}}{C}-N-(CH3)2$$
$$\underset{\displaystyle \underset{\displaystyle N-(C H3)2}{|}}{\overset{\displaystyle |}{C = O}}$$

-98-

59. A method of claim 57, wherein the compound has a structure:

$$CH_3-N-C-(CH2)y-A_1-(CH_2)Y_1-C-N-CH_3$$

(with O double-bonded above each C, and CH$_3$ branches below each N)
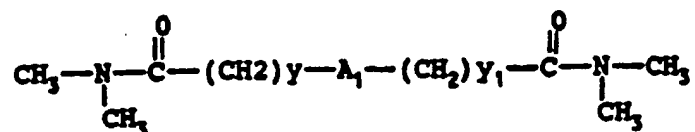
60. A method of treating patient having a tumor characterized by proliferation of neoplastic cells which comprises administering to the patient an amount of a compound effective to selectively induce terminal differentiation, which neoplastic cells and thereby inhibit their proliferation, the compound having the structure:

$$HOHN-C(-(X)_n-)C-NHOH$$

(with O double-bonded above each C)
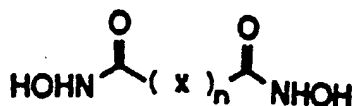
wherein X is phenyl or methyl and n is an integer from 1 to 15.

61. A method of treating patient having a tumor characterized by proliferation of neoplastic cells which comprises administering to the patient an amount of a compound of claims 23-39 in an amount effective to selectively induce terminal differentiation of neoplastic cells and thereby inhibit their proliferation.

62. A method of claim 60 or 61, wherein the amount of the

-99-

compound is less than an amount which causes toxicity in the patient.

63. A method of claim 62, wherein the tumor is lung cancer, acute lymphoid myeloma, bladder melanoma, renal carcinoma, breast carcinoma or colorectal carcinoma.

64. A method of claim 60 or 61, wherein the administering is effected intravenously.

65. A method of claim 60 or 61, wherein the administering is effected orally.

66. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the compound of claim 1 or 23-39, in an amount effective to selectively induce terminal differentiation of suitable neoplastic cells and less than an amount which causes toxicity in a patient.

67. A pharmaceutical composition of claim 66, wherein the amount of the compound is from about 0.1 gm/m^2/day to about 30 gm/m^2/day.

68. A pharmaceutical composition of claim 66, wherein the compound has the structure:

$$(CH_3)_2-N-\overset{\overset{O}{\|}}{C}-(CH_2)_5-\overset{\overset{\overset{CH_3CH_2O}{\diagdown}\overset{O}{/\!/}}{\diagup\!\!\diagdown C}}{\underset{\underset{O\diagup}{\overset{|}{C}}\diagdown}{\underset{OCH_2CH_3}{|}}}-(CH2)_5-\overset{\overset{O}{\|}}{C}-N-(CH_3)_2$$
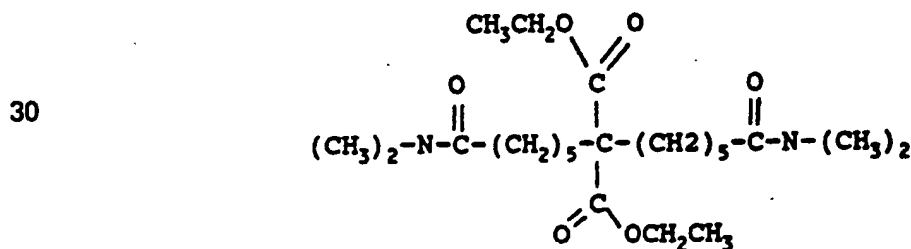
69. A pharmaceutical composition of claim 66, wherein the

-100-

amount of the compound is not more than about 30 gm/m^2/day.

70. A pharmaceutical composition of claim 66, wherein the compound has the structure:

$$(CH_3)_2-N-\overset{\overset{\textstyle O}{\|}}{C}-(CH_2)_5-\overset{\overset{\textstyle H}{|}}{\underset{\underset{\textstyle N-(CH3)_2}{|}}{\underset{\textstyle C=O}{|}}}{C}-(CH_2)_5-\overset{\overset{\textstyle O}{\|}}{C}-N-(CH_3)_2$$
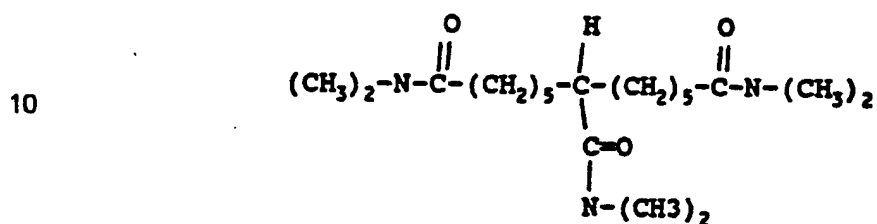
71. A pharmaceutical composition of claim 70, wherein the amount of the compound is not more than about 30 gm/m^2/day.

72. A pharmaceutical composition of claim 66, wherein the compound has the structure:

$$H-N-(CH2)_6-N-(CH_2)_6-N-H$$
$$\underset{\underset{\textstyle CH_3}{|}}{\underset{\textstyle C=O}{|}} \quad \underset{\underset{\textstyle CH_3}{|}}{\underset{\textstyle C=O}{|}} \quad \underset{\underset{\textstyle CH_3}{|}}{\underset{\textstyle C=O}{|}}$$
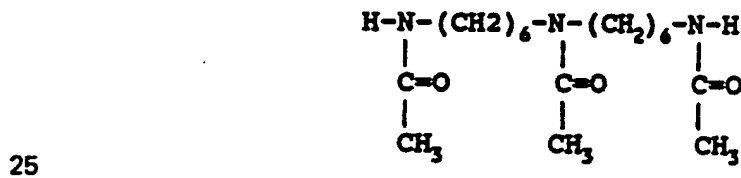
73. A pharmaceutical composition of claim 72, wherein the amount of the compound is not more than about 30 gm/m^2/day.

FIGURE 1 A

A

BENZIDINE REACTIVE CELLS (%)

CONCENTRATION
(mM)

FIGURE 1 B

A

CONCENTRATION

(mM)
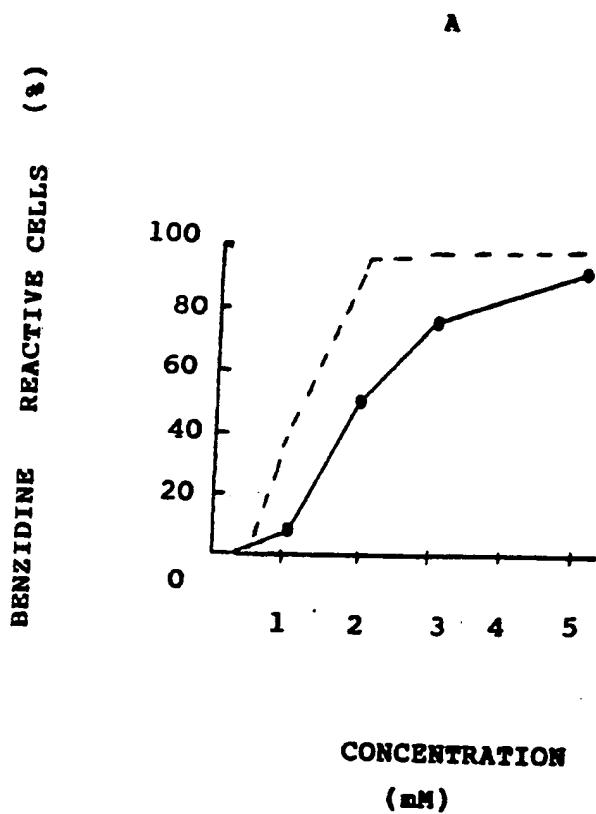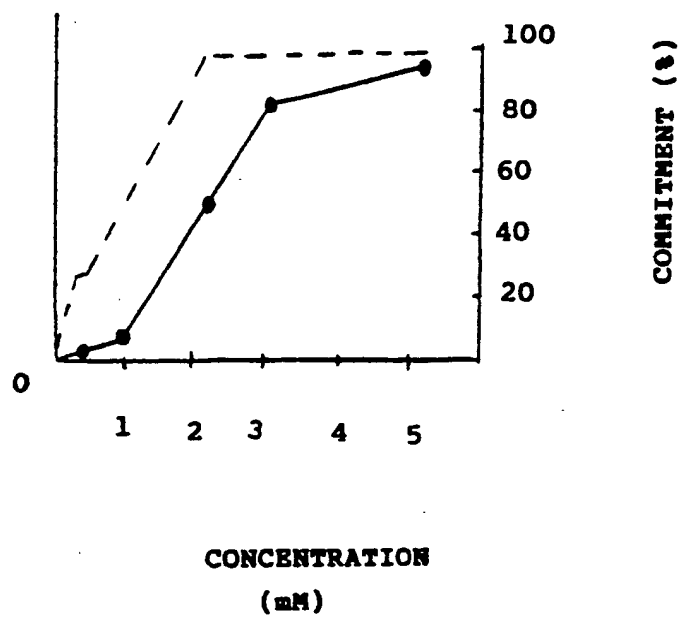
FIGURE 1 C

B

BENZIDINE REACTIVE CELLS (%)
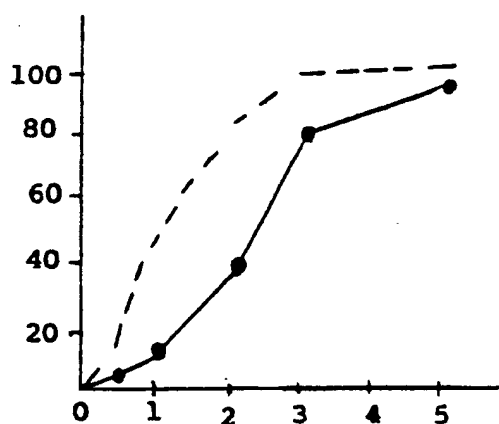
CONCENTRATION

(mM)

FIGURE 1 D

B

CONCENTRATION

(mM)

FIGURE 1 E

C

CONCENTRATION (mM)

FIGURE 1F

C

CONCENTRATION   (mM)

FIGURE 2A

CONCENTRATION

(mM)

FIGURE 2B

CONCENTRATION

(mM)
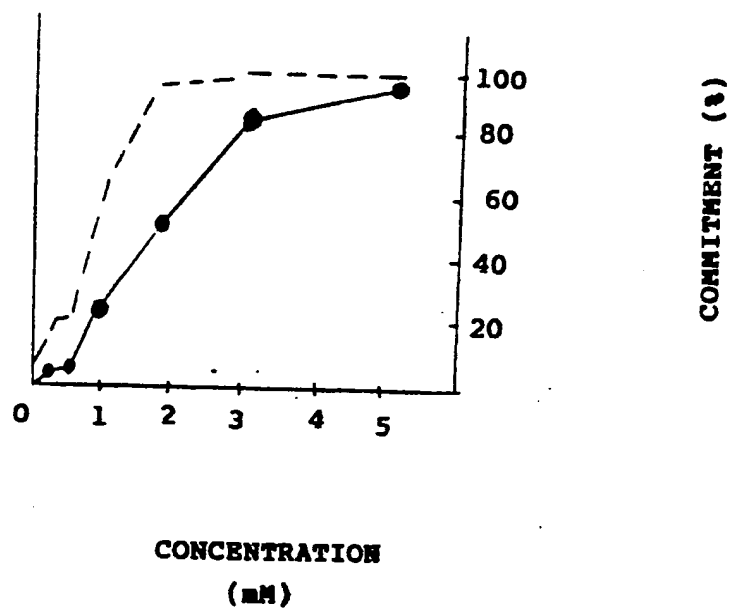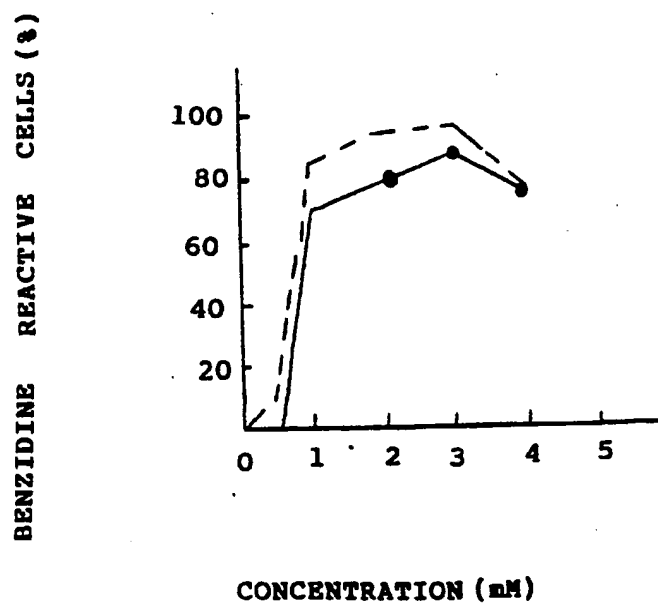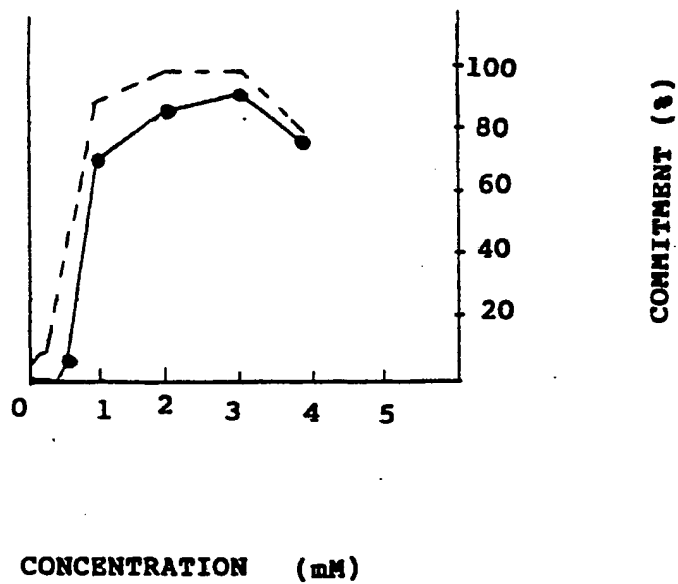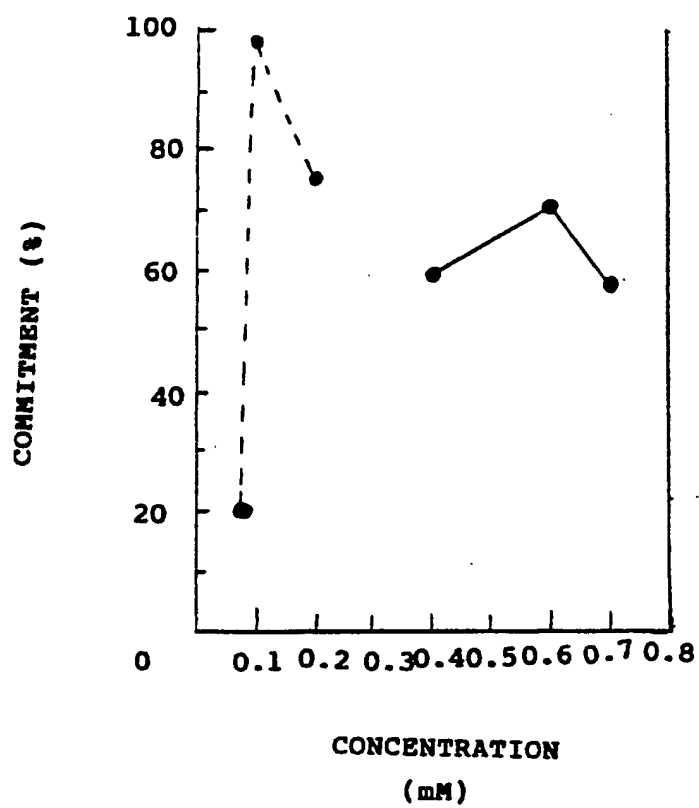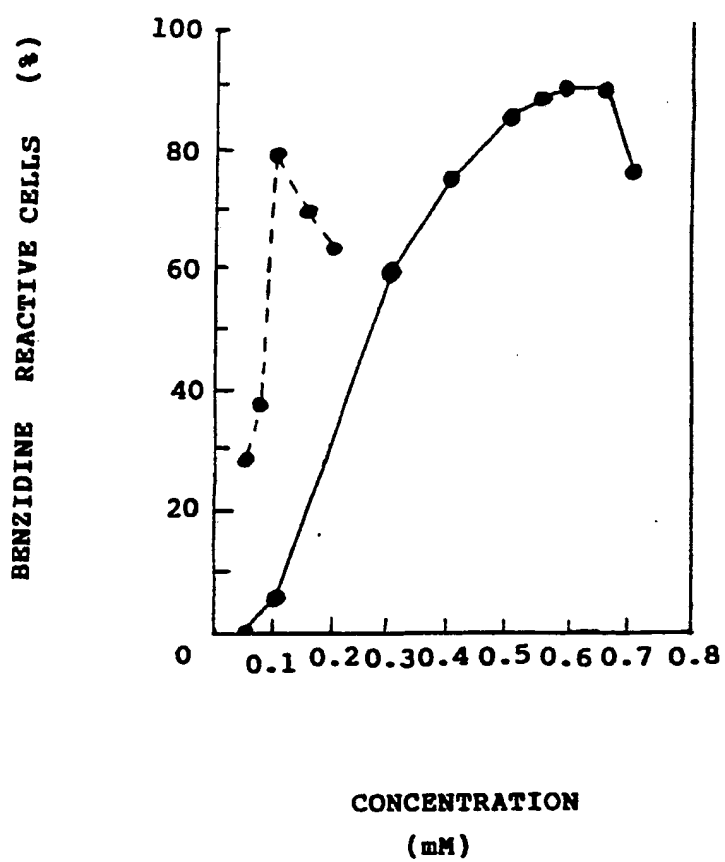
FIGURE 3A

OPTIMAL CONCENTRATION OF IC-135 FOR
INDUCEMENT OF TERMINAL DIFFERENTIATION

K-135 conc.	cell count	B+ cells
1mM	2.4×10^6	68-69
2mM	1.9×10^6	77-80
3mM	1.2×10^6	78-81
4mM	0.5×10^6	3-4
5mM	0.2×10^6	0-1

Control cell $\longrightarrow$ 0-1

5mM HMBA $\longrightarrow$ 89-88

FIGURE 3B

OPTIMAL CONCENTRATION OF IC-135 FOR
INDUCEMENT OF TERMINAL DIFFERENTIATION

5mM HMBA   ——————>   89-88

[2-3mM is optimal]

FIGURE 4

COMPARISON OF HMBA AND IC-135 ON DS19 CELLS

legend: +++ contr l   --- IC-135   —— HMBA
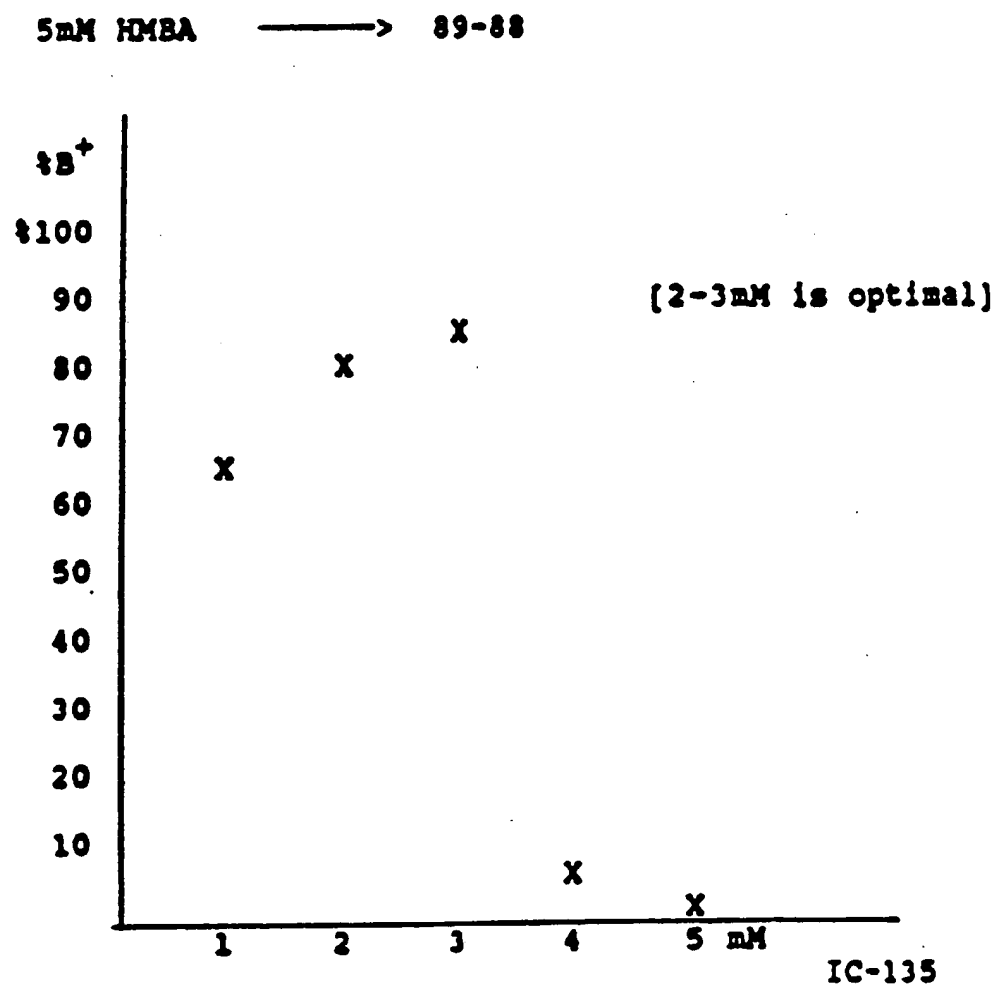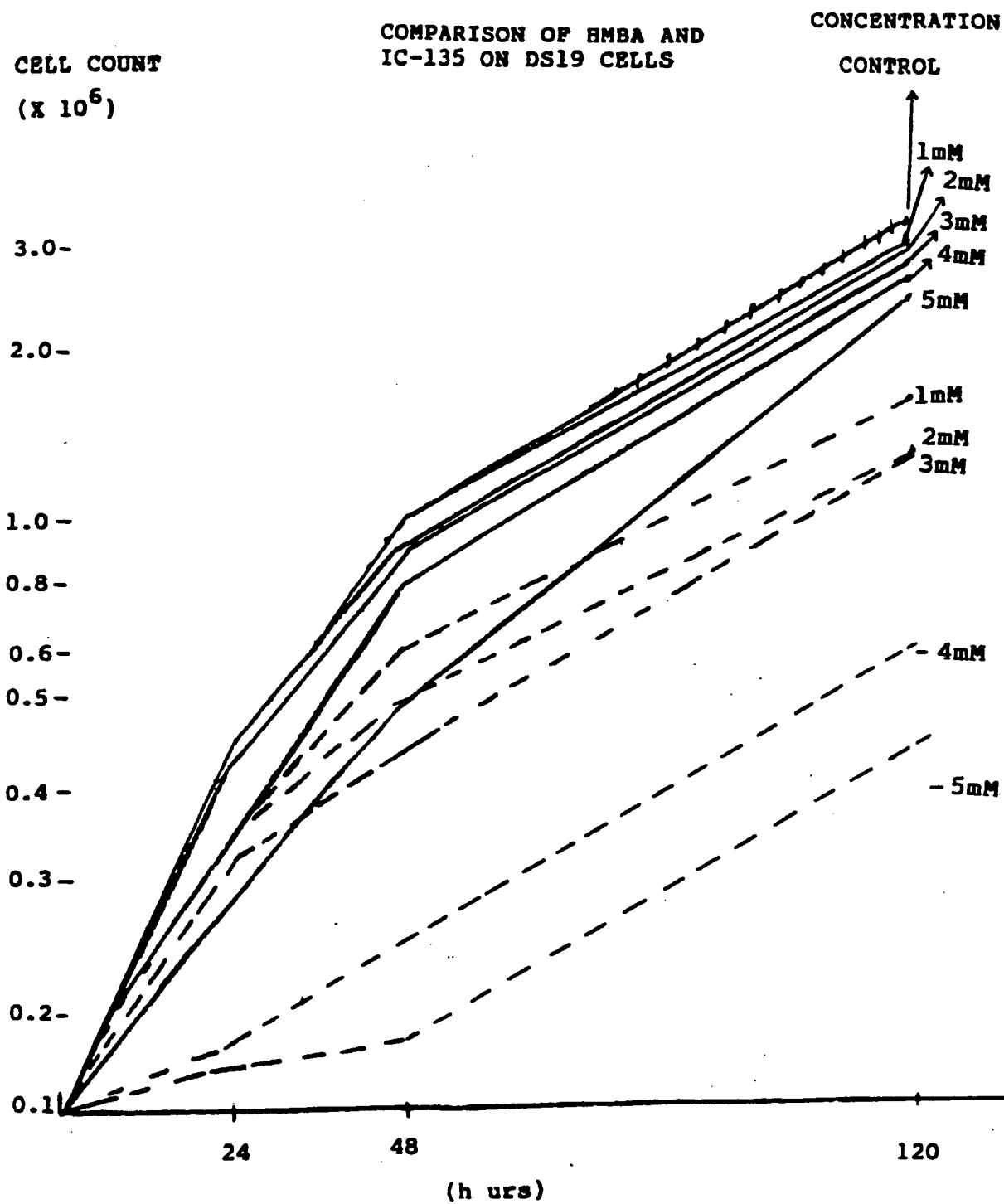
FIGURE 5A

COMMITMENT (%)   V 3-17
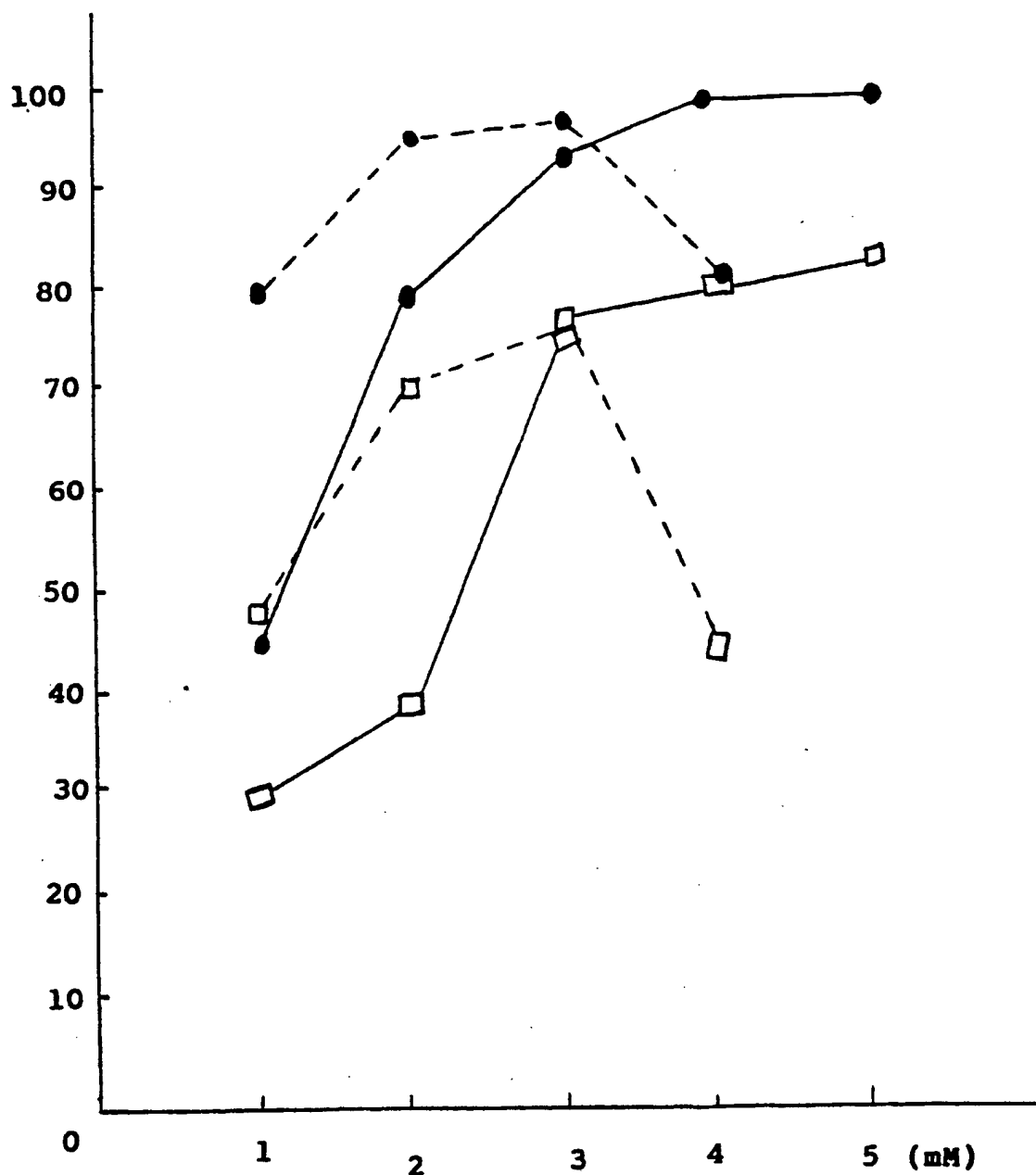
LEGEND: IC-135 @ 24 HRS.□--□   HMBA @ 24 HRS. □——□

          IC-135 @ 48 HRS.●--●   HMBA @ 48 HRS. ●——●

FIGURE 5B

COMMITMENT (%)
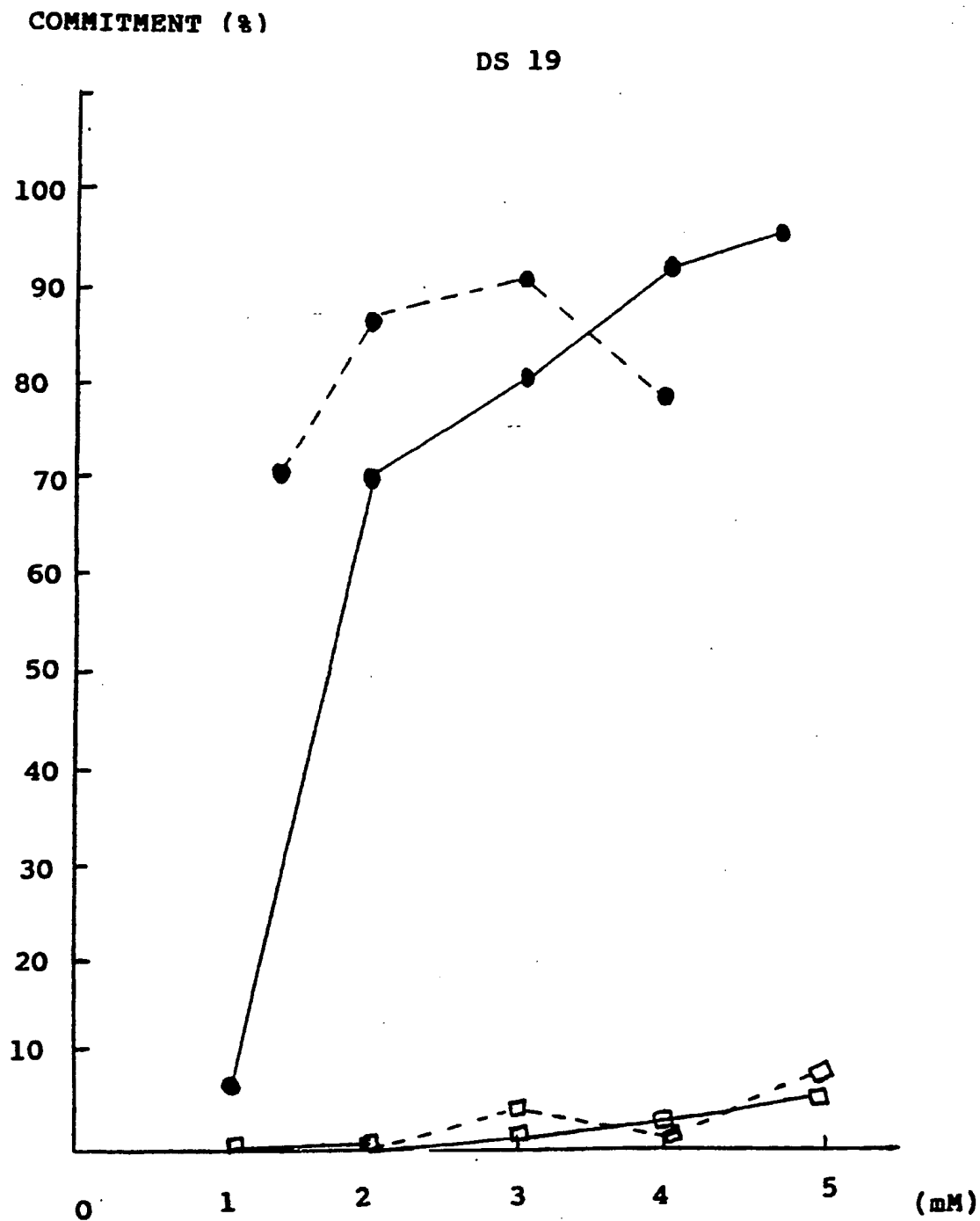
DS 19

LEGEND: IC-135 @ 24 HRS.□--□ HMBA @ 24 HRS. □———□

IC-135 @ 48 HRS.●--● HMBA @ 48 HRS. ●———●

FIGURE 6A

V3-17

LEGEND: HMBA ▮   IC-135 ▯

FIGURE 6B

DS-19

CONCENTRATION
(mM)
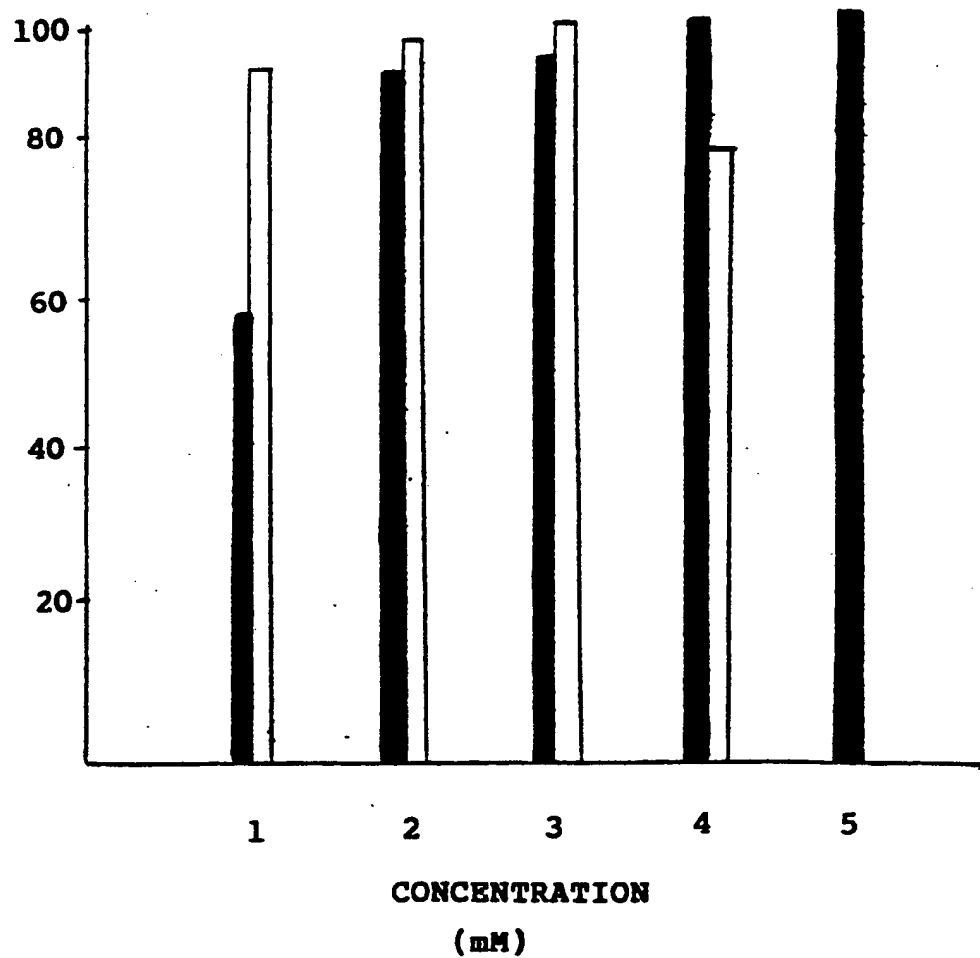
LEGEND: HMBA ■   IC-135 ▯
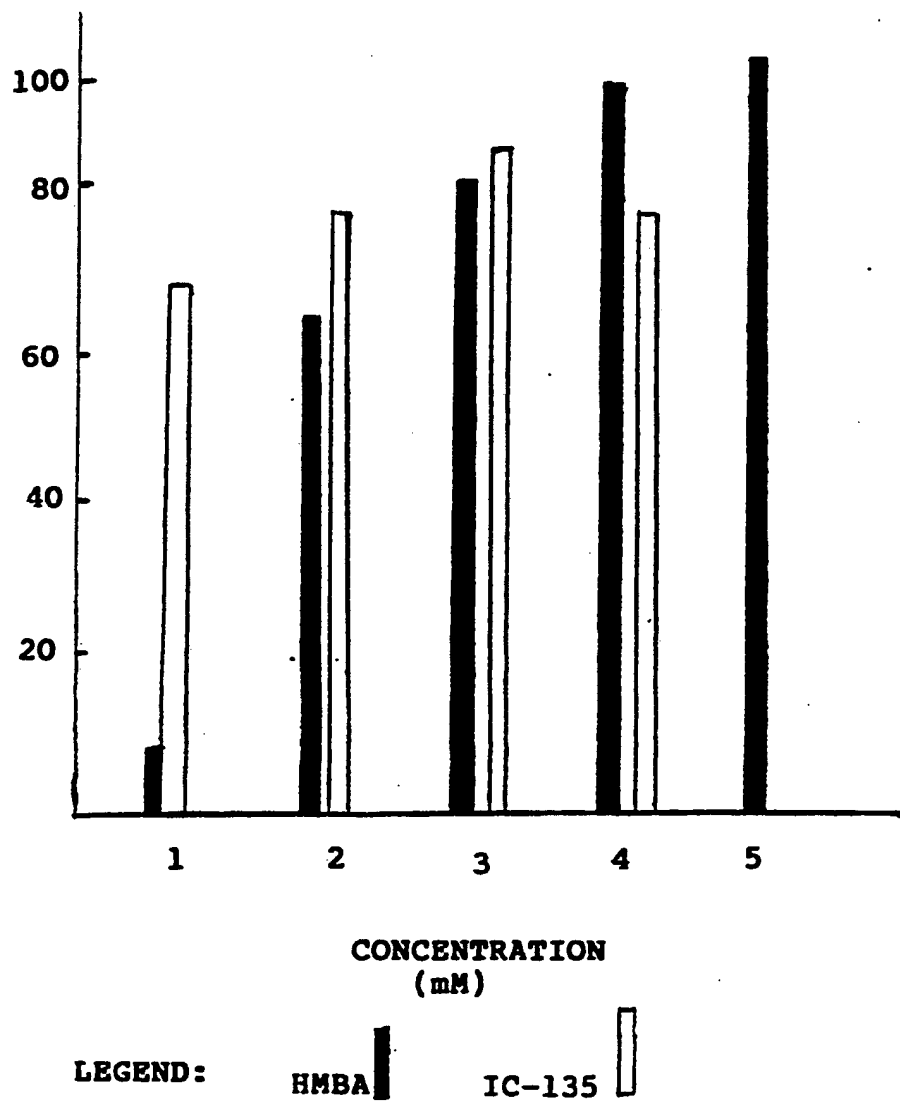
INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/06649

I. CLASSIFICATION F SUBJECT MATTER (if several classification symbols apply, indicate all) ³

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C07C 69/34 229/24, 233/04 235/20; A61K 31/22
U.S. CL: 169, ⁻⁻¹; 514/547,551,552 564/152,153

II. FIELDS SEARCHED

Minimum Documentation Searched ⁴

Classification System	Classification Symbols
U.S. CL:	560/169,171 564/152,153 514/547,551,552

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁶

STN CAS online U.S. Parent prior art 270,963
APS   374,343

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

Category *	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
P,Y	US, A, 4,801,748 (MURAHASHI ET AL) 31 January 1989 p5, No. 17	1,3-16
Y	US, A, 4,442,305 (WEITLE ET AL.) 10 April q984 see entire document.	1,3-16
Y X	Arneim-Forsch Vol 35, No 7, "Quantitative analysis of biological activities of diactinomyein analogs and methotrexate derivatives with Vander Waals volume" PRABHAKER ET AL, 1985 pages 1030-3.	1,3-5,7,10-12 36 and 38
Y, A X	Int. J. Cancer Vol 23, No 1, "Induction of Erythroid differentiation in murine erythroleukemia cells by nitrogen substituted polymethylene diamides", HOZUMI et al pp 119-22	1,3-5,7,10-12 40,42,49,51, 53,66,67,69 36 and 38

* Special categories of cited documents: ¹⁵

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search ⁸	Date of Mailing of this International Search Report ⁸
23 APRIL 1991	0 3 MAY 1991

International Searching Authority ¹	Signature of Authorized Officer
ISA/US	BRIAN G. BEMBENICK

Form PCT/ISA/210 (second sheet) (May 1986)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	"Organic Chemistry:, Morrison and Boyd, 3rd Ed. Allyn and Baron Inc. p 755	1,3-16
Y/A	Proc. Natl. Acad. Sci. USA, Vol 72 No 3 "Induction of Erythroid Differentiation ..." etc. TANAKA et al, March 1975, pp 1003-6	40,42-49,51,52 / 41
	J. Biol. Chem. Vol 253, No 12 "Inducers of Erytholeukemic Differentiation", REUBEN et al, June 1978 pp 4214-18	40,42-49,51,52 / 41
A	J. Org. Chem. Vol 46, No 25, "Lipophilic enterobactin analogs", WEITL et al, 1981, pp 5234-7	1,13,16

V.☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1.☐ Claim numbers . . . . . . because they relate to subject matter [1] not required to be searched by this Authority, namely:

2.☐ Claim numbers . . . . because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out [1], specifically:

3.☐ Claim numbers . . . . because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI.☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

I. Claims 2,24,37,39,41 and 68 drawn to Ester compounds (class 560) compositions and Method of Use I.

II. Claims 13-16,19,20,22,29,36,38,43-48,70-73 drawn to Amide compounds (564/152) compositions and Method of Use I.

1.☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.

2.☒ As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:
Group I 2, 24, 37, 39, 41, 68; Group II 13-16, 19-20, 22, 29, 36, 38, 43-48 and 70-73 and the generic claims as they read on the elected groups clais 1, 3-12, 17, 18, 21, 27, 35, 66, 67, 69, 40, 42, 49, 51 and 52

3.☐ No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4.☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

CONTINUATION SHEET

III. Claims 23,25 and 34 drawn to Barbituric acid compounds (class 544/301), compositions and Method of Use I.

IV. Claim 26 drawn to bis - Barbituric acid compounds (class 544/296), compositions and Method of Use I.

V. Claims 28,33 and 50 drawn to Hydroxamic acid compounds (class 564/160), compositions and Method of Use I.

VI. Claims 30 drawn to Hydrazide compounds (class 564/148), compositions and Method of Use I.

VII. Claim 31 drawn to Pyrazolidine dione compounds (class 548/361), compositions and Method of Use I.

VIII. Claim 32 drawn to seven-membered heteroring compound (class 540/492), compositions and a Method of Use I.

IX. Claims 2,24,37,39,54 and 68 drawn to Ester compounds (class 540/560), compositions and Method of Use II.

X. Claims 13-16,19,20,22,29,36,38,54-58 and 70-73 drawn to amide compounds (class 564/152), compositions and Method of Use II.

XI. Claims 23,25 and 34 drawn to Barbituric acid compounds (class 544/301), compositions and Method of Use II.

XII. Claim 26 drawn to bis - Barbituric acid compounds (class 544/296), compositions and Method of Use II.

XIII. Claims 28,33 and 60 drawn to Hydroxamic acid compounds (class 564/160) compositions and Method of Use II.

XIV. Claim 30 drawn to Hydrazide compounds (class 564/148), compositions and Method of Use II.

XV. Claim 31 drawn to Pyrazolidine dione compounds (class 548/361), compositions and Method of Use II.

XVI. Claim 32 drawn to seven-membered heteroring compounds (class 540/492), compositions and Method of Use II.

Generic Claims:
    1) Compounds 1,3-12,17,18,21,27,35,66,67,69

    2) Method I 40,42,49,51,52
    3) Method II 53,59 and 61-65